THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

ORDINARY MEETING

AN Ordinary Meeting of the Society was held on Thursday and Friday, October 5th and 6th, 1961, at The Institution of Mechanical Engineers, Birdcage Walk, London, S.W.1. The Chair was taken by the President, Dr. A. J. Amos, F.R.I.C.

The following papers were presented and discussed: "Studies of the Boron - Curcumin Complex and its use in Trace Boron Analysis," by M. R. Hayes, A.R.I.C., and J. Metcalfe, B.Sc.; "Applications of Micro-coulometry," by R. G. Monk, A.R.C.S., D.I.C., Ph.D., K. C. Steed and G. C. Goode, B.Sc., A.R.I.C.; "The Gravimetric Determination of Uranium as the Phosphate," by J. S. Wright, J. A. Ryan, A.R.I.C., and T. J. Hayes, A.R.I.C.; "A Study of the Determination of Oxygen in Beryllium by Vacuum Fusion," by M. R. Everett and J. E. Thompson; "The Application of the Conductimetric Method for the Determination of Carbon to Highly Alloyed Steels and the Less Common Metals," by J. E. Still, B.Sc., F.R.I.C., and I. R. Green, A.R.I.C.; "The Identification and Determination of Foreign Phases and Constituents in Metals, with Special Reference to Beryllium," by H. P. Rooksby, B.Sc., F.Inst.P., and I. R. Green, A.R.I.C.; "Developments in Emission Spectrography Arising from the Routine Determination of the Isotopic Abundance of Uranium," by R. Franklin and J. R. Hartley, B.Sc., A.R.I.C.; "Suspension Scintillation Counting of Carbon-14 Barium Carbonate," by H. J. Cluley, Ph.D., F.R.I.C.; "The Use of Isotope Dilution for the Determination of Hydrogen in Metals, with Particular Reference to Alkali Metals," by C. Evans, B.Sc., Dip.Chem.Eng., and J. Herrington, B.Sc.; "Reverse-phase Partition Chromatography," by T. J. Hayes, A.R.I.C., and A. Hamlin, B.Sc., F.R.I.C.; "Gas Chromatography in the Analysis of Inorganic Systems," by T. R. Phillips, Ph.D., B.Sc., and G. Iveson; "The Analysis of Nuclear Reactor Carbon Dioxide for Gaseous Impurities," by R. M. S. Hall, M.A., A.R.I.C., A.Inst.P.

DEATH

WE record with regret the death of

Thomas Cockburn.

Obituary

HAROLD WRIGHT HODGSON

HAROLD WRIGHT HODGSON died on May 13th last, in his forty-eighth year. His sudden and untimely death, at the peak of his career, was a severe shock to his many friends.

Hodgson spent his early life in the North-West of England and was educated at Arnold House, Blackpool. His chemical training was obtained at Liverpool Technical College, and he received his first experience in industrial analysis in the laboratories of William Crawford and Sons Limited, Liverpool.

In 1940 Hodgson joined the staff of Roche Products Limited, at Welwyn Garden City, where he stayed until 1946. He developed a keen interest in pharmaceuticals during this time, which he maintained throughout his career.

He joined the newly formed Research and Development Department of The British Oxygen Company Limited in 1946, to start the Analytical Laboratory. This he developed into one of the most modern laboratories in the country, and latterly his duties involved the co-ordination of Analytical Services for the whole of The British Oxygen Organisation.

Hodgson took a special interest in gas analysis and represented the Association of British Chemical Manufacturers on the B.S.I. Committee on "Sampling and Analysis of Gases"; he represented our Society on the B.S.I. Committees on "Paramagnetic and Infra-Red Methods" and on "Gas Chromatography." He was a keen member of the Society and served on the Council in 1953 and 1954.

Hodgson had a love for cars and motoring; his mechanical skill was considerable, and he was a willing helper at motor racing meetings. This interest no doubt contributed to the great success of the first Rally held by the London Section of the Royal Institute of Chemistry, of which he was one of the organisers.

Harold Hodgson was unmarried; he had a very wide circle of friends, all of whom knew him as a most generous and hospitable man.

J. H. GLOVER

The Identification of Small Amounts of Bases in Urine by Infra-red Spectrophotometry

By P. J. MORGAN

(Department of Pharmacology, University of Melbourne, Australia)

A method is described for the rapid extraction of organic bases from urine, in amounts of the order of 100 μg, in a sufficiently pure condition for identification by infra-red absorption spectrophotometry.

THE analytical significance of all organic compounds having highly characteristic absorption spectra in the wavelength range between 2·5 and 15 μ has, in recent years, resulted in the availability of instruments that record spectra in this range and yet do not call for a prohibitively large capital outlay in view of the useful work they can perform.

An objection often made when considering the application of infra-red absorption to substances extracted from biological sources is that any associated impurities will contribute absorption bands similar in intensity to those given by the substance being studied. The extraction procedure described here is sufficiently selective when applied to urine to permit this disadvantage to be overcome.

The procedure is an adaptation of the acid-spot extraction method previously described[1] for detecting organic bases in urine at the 1 p.p.m. level. In this method, bases are transferred from a chloroform extract of the urine to spots of dilute sulphuric acid supported on strips of Whatman 3MM filter-paper and are detected by paper chromatography of the strips in acidified solvents after exposure to ammonia vapour.

The procedure described here supplements this method in that a larger amount of any base detected on the paper chromatograms can be rapidly isolated in a relatively pure condition. It also facilitates the rapid detection of bases, such as morphine, not readily soluble in organic solvents, when these are present in small concentrations in the urine.

METHOD

APPARATUS—

A modified form of the acid-spot extraction apparatus described previously is used (see Fig. 1). It consists essentially of an inner jacket conveniently made from a Liebig condenser 1½ inches in diameter and cut off square near one end. It should be made as long as possible, but should still maintain reasonable rigidity of the central tube; a length of 12 inches was found to be satisfactory. The tip of the central tube is constricted in a flame to give an opening about ⅛ inch in diameter. The open end of the Liebig jacket fits into a cylindrical beaker 1½ to 1¾ inches in diameter and 7½ inches tall, which is supported in a water bath. The outlet at the top of the Liebig jacket is connected by means of an 18-inch length of polythene tubing to a test-tube fitted with a side-arm. This serves as a trap for condensed solvent vapours and may be cooled if desired.

An acidified solution of chromous sulphate, concentrated sulphuric acid and a U-tube of silica gel may be used as previously described[1] to provide a dry stream of nitrogen. If the gas is passed through a U-tube of fresh self-indicating silica gel, this will change in colour from pink to blue if the gas is being satisfactorily dried.

ACID-SPOT EXTRACTION PROCEDURE—

A chloroform extract of the urine is prepared by gentle agitation of the specimen with 100 ml of chloroform after adjustment of pH to a suitable value with 20 per cent. sodium hydroxide solution as previously described.[1] The chloroform extract is introduced into the beaker, which is supported in the water bath at a temperature at least 20° C above that of the room.

Spots of 0·25 and 0·5 N sulphuric acid - thymol blue reagent[1] are placed about 3 cm from one end of a strip of Whatman 3MM filter-paper having the same width as the Liebig jacket and of any desired length. When the spots have dried somewhat, the strip is inserted into the Liebig jacket, which is then clamped in the beaker at a slight angle to the vertical. The angle of tilt and the arrangement of the polythene tubing should be such that any solvent

condensing in the polythene tube does not run back into the chloroform extract. The acid spots should be below the surface of the chloroform extract, which is agitated by passing a brisk stream of dry oxygen-free nitrogen through the central tube of the jacket. Two filter-paper strips may be inserted if so desired, one on each side of the central tube.

Extraction of bases proceeds on to the acid spots, which are examined from time to time for any change in colour of the thymol blue indicator. If the red colour of the spot of 0·25 N acid shows any change towards neutrality (yellow) when compared with the spot of 0·5 N acid, the strip is immediately removed and replaced by a fresh strip. Generally, any early tendency to neutralisation is rapidly overcome as volatile bases, such as ammonia and amines having low molecular weights, are swept out of the chloroform extract by the stream of gas. The tendency of chloroform to emulsify in the urine generally makes a second extraction with a further portion of chloroform desirable; further, for a base not particularly soluble in chloroform, several successive extractions may be needed to extract most of the base. These portions of solvent can be added via the side of the beaker from time to time as chloroform evaporates into the stream of nitrogen and serve to replenish base that is being removed from the solution on to the acid spots.

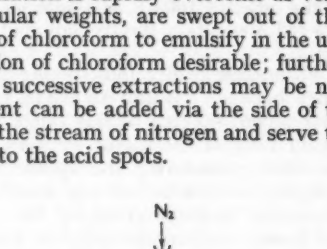

Fig. 1. Acid-spot extraction apparatus

Any thick emulsion remaining after the final extraction with chloroform can be broken as follows.

The emulsion is run, in small portions, from a separating funnel into a flask and then shaken vigorously. If the proportion of non-aqueous phase is sufficiently high, the shaking will break the emulsion. When this procedure is no longer effective, small amounts of cyclohexane are added to the emulsion, and the vigorous shaking is repeated; any further organic phase that separates is added to the main extract in the acid-spot extraction apparatus (with filtration through a dry filter-paper if it is turbid). As a last resort, vigorous shaking, together with the addition of small amounts of anhydrous sodium sulphate, will result in further breaking of the emulsion.

Since cyclohexane is a much poorer solvent than chloroform and is also appreciably less volatile, its introduction into the extraction system will lead to an increase in the rate of extraction of most bases on to the acid spots in the final stages. The lack of solubility of some extremely weak bases, such as caffeine, in cyclohexane can be utilised to achieve acid-spot extraction of these bases when they occur in the urine at concentrations of about 1 p.p.m.

It is preferable to pass nitrogen until the volume of chloroform has been decreased to about 50 ml and then to dilute with cyclohexane to about 200 ml. The passage of gas is continued until the chloroform content of the extract has been decreased to less than 1 per cent. by volume; this operation is conveniently carried out overnight at 45° C.

The chloroform content is easily determined by recording an infra-red spectrum in a cell 0·05 mm thick, when the intense chloroform absorption band at 13·1 μ can be used to determine concentrations of chloroform of less than 1 per cent., the weaker band near 8·3 μ in the range 1 to 10 per cent. and the weak cyclohexane band near 8·0 μ when the chloroform content is more than 10 per cent. by volume.

PAPER CHROMATOGRAPHY OF ACID-SPOT EXTRACTS—

Chromatography of the strips removed from the acid-spot extraction apparatus can be carried out as previously described[1] by exposing them to ammonia vapour for a short time to neutralise the excess of sulphuric acid on the spots and then developing in either the n-butyl alcohol - hydrochloric acid or the isobutyl methyl ketone - acetic acid solvent. These solvents were chosen for the rapid detection of bases because they give good chromatograms without the need for any equilibration; however, for purposes of isolation, it is preferable to allow some time for equilibration, particularly if the thick Whatman 3MM filter-paper is used.

Detection of typical alkaloids is achieved, as before, by spraying the chromatograms with the iodoplatinate reagent. For the detection of the weak base caffeine it is essential to add about 6 per cent. of concentrated hydrochloric acid to this reagent. Amines of lower molecular weight, which may not react satisfactorily with the iodoplatinic acid to give the coloured precipitate on the paper, are detected by spraying with bromocresol green in ethanol after development in the isobutyl methyl ketone - acetic acid solvent.

A base may be recovered in good yield from chromatograms that have been sprayed with either or both of these reagents by cutting out the coloured spots, placing them in a small Erlenmeyer flask (Quickfit & Quartz Ltd.) and adding sufficient concentrated ammonia solution just to moisten the paper completely. The stoppered flask is shaken in a mechanical shaker for 30 minutes with sufficient chloroform just to cover all the pieces of paper (the shaking should not be so vigorous that the paper disintegrates). If this condition is observed, it is possible to check for completeness of extraction of base by drying the pieces of paper and re-spraying them with the iodoplatinate reagent after decantation of the chloroform extract. If any cellulose fibres are detached by over-vigorous shaking, or if too much ammonia has been used, then the extract must be filtered, and completeness of extraction checked by re-shaking the residue *plus* the filtering medium with a further amount of chloroform. Prolonged shaking of the initial extract should be avoided, as it may lead only to a higher level of impurities in the extract. When this procedure is adhered to, it has been found that bases are obtained in a sufficient degree of purity to give a useful infra-red spectrum; it is best to aim at the collection of a few hundred micrograms of base from about ten chromatographic spots.

INFRA-RED SPECTROPHOTOMETRY OF EXTRACTS—

Spectrophotometry is best carried out in solution in an organic solvent in micro cells having sodium chloride windows. The chloroform extract from the chromatographic spots must be evaporated to dryness before double-beam spectrophotometry can be attempted, even if chloroform is the solvent of choice. This is conveniently carried out in a glass tube having a narrow tip, such as a centrifuge tube, by passing a stream of nitrogen through the extract at 40° C. Vapour condensing on the cooler sides of the tube eventually washes all the solute down into a small region at the narrow tip, so that, when the amount of solvent required to fill the micro cell is added, the solute is totally covered. Solution may be assisted by the application of gentle heat to the tip of the tube if precautions are taken to avoid loss of solvent by evaporation.

After a spectrum has been recorded, some sort of purification technique should be applied to the sample. This purification may take the form of a further paper-chromatographic stage if the level of impurities appears to be high. It is preferable to use a solvent system different from that used for developing the first chromatograms of the acid spots. Infra-red absorption bands that disappear or are decreased in absorption relative to other bands in

the spectrum after the purification can then be ascribed to the impurities present. When paper chromatography is used, it is never possible to get rid of the last traces of absorption (due to organic impurities from the paper) in the carbon - hydrogen stretching and bending regions of the spectrum. Sublimation *in vacuo* is often a most effective final procedure for purification.

The use of a double-beam spectrophotometer recording from 2·5 to at least 15 μ is essential for the satisfactory study of the small amounts of material being examined. Instruments that cover this range are usually fitted with a sodium chloride prism for dispersing the radiation; however, an instrument having a diffraction grating for dispersing the radiation is generally capable of giving better results in the shorter-wavelength portion of the spectrum. A choice of two widely different recording speeds is desirable, as this permits more careful coverage of spectral regions in which absorption from the solvent is high. To cover regions in which absorption from the solvent is extremely high, the use of an alternative solvent is necessary.

A laboratory-reagent grade of tetrachloroethylene (fractionated before use, with rejection of the initial and final fractions) was found to be most satisfactory for work on the micro scale; this solvent keeps well in a dark-glass bottle. When a fairly large amount of material has been isolated in a substantially pure condition, simple washing of the sample with small portions of tetrachloroethylene may remove sufficient of the impurities present to permit detection of the absorption bands associated with the substance under investigation. It is recommended that micro cells about 0·3 and 1·0 mm thick should be used for work with tetrachloroethylene. It should be noted that the extraction method described is biased in favour of substances readily soluble in organic solvents and that thicker cells may be required for some solutes. When lack of solubility in organic solvents is associated with the presence of hydroxyl groups in the molecule, it is sometimes expedient to improve the solubility by partial acetylation of the substance. For example, it was found that conversion of morphine into its monoacetyl-derivative permitted a much more detailed spectrum to be obtained in a cell 0·3 mm thick than could be obtained from morphine itself in a cell ten times as thick. The fact that hydroxyl groups can form intermolecular hydrogen bonds, even in fairly dilute solution, should be kept in mind when two spectra are being compared, as, when such bonds are formed, the sharp absorption band due to free hydroxyl is replaced by a much broader band at a longer wavelength. Some general aspects of infra-red solvents have been discussed by Cole.[2] The use of tetrachloroethylene has been discussed elsewhere.[3]

Discussion of the method

The extraction method described is based on a 500-ml sample of urine, but it can be applied as it stands to larger or smaller samples, within reason. Up to 5 per cent. of ethanol may be added to the chloroform used for extraction of the urine in order to improve the rate of extraction of substances, *e.g.*, morphine, soluble only with difficulty, but it is doubtful whether this offers any real advantage, since not only are more impurities extracted from the urine, but difficulty may also be caused by emulsification of solvent in the urine.

In order to minimise loss of thymol blue indicator from the acid spots, it is advisable that they should be allowed to dry in air before introduction into the chloroform extract; however, it should be realised that the spots will pick up any volatile bases present in the laboratory atmosphere if they are left exposed for too long a period. Since the thymol blue indicator is retained near the centre of the spot when the acid spots are applied to the paper, it is possible to follow the progress of extraction by adding a drop of an extremely dilute solution of the basic dye methylene blue in ethanol to the chloroform extract. The extracted dye also serves as a standard of R_F value when the extracts are subjected to chromatography. Since the colour of the thymol blue indicator may occasionally not be visible when the strips are removed, a fairly concentrated solution of thymol blue in ethanol should be kept at hand and applied to the spots with a loop of platinum wire if there is any doubt. Unless the spots have retained their acidity to thymol blue, there is no guarantee that weak bases initially extracted on to the spots have not been displaced by stronger bases.

If long strips of paper are used in the apparatus, it is essential to keep the chloroform extract at a temperature substantially higher than that of the room so that some condensation of solvent vapour will occur on the portion of the strip not immersed in the solvent. This serves to keep the upper part of the strip washed free of solutes that may otherwise accumulate

there. If short strips completely immersed in the extract are used, it is not necessary to apply heat, and the risk of loss of any labile material is therefore substantially decreased; however, the time needed for extraction is increased, and chromatography of short strips presents some difficulties, as it is necessary to place the spots of acid close to the end of the strip, and this results in too fast a flow of solvent over the spots when chromatography is begun.

This defect may be overcome in three ways, as shown in Fig. 2. The end of the strip may be cut into a serrated pattern, with the tips of the serrations just touching the solvent when chromatography is begun, or wire staples may be used to attach another piece of paper to the end of the strip to serve as a "lead-up" for the solvent. Alternatively, if the highest possible purity is to be sought from the chromatogram, a small rectangle containing the spots

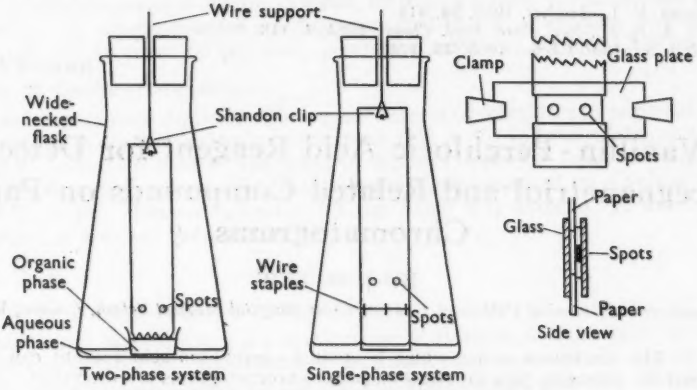

Fig. 2. Some methods for paper chromatograms

is cut out and placed centrally between two glass plates. One edge of the assembly is grasped between thumb and fore-finger so that the other edge opens out slightly, with the edge of the paper as fulcrum, and a short "lead-up" length of paper is inserted to meet the rectangle, with a slight overlap. The pressure is then transferred to the other side of the assembly to permit similar insertion of a longer piece of paper on the other side of the rectangle. The assembly is then clamped together with clothes pegs of the spring-type, and chromatography is carried out by the ascending-solvent technique.

TABLE I

LIMITS OF DETECTION OF VARIOUS BASES IN URINE

Base	Limit of detection (as concentration in original sample) in— initial chloroform extract at pH 9, p.p.m.	solution after concentration of several extracts to 50 ml, p.p.m.	cyclohexane solution containing 1% v/v of chloroform, p.p.m.
Strychnine	2	1	$\leqslant 1$
Cocaine	2	1	< 1
Amphetamine	5	2	1
Morphine	8	2	1
Caffeine	100	40	2

In the final stages of the acid-spot extraction from the cyclohexane-rich solvent, some solid matter may be deposited near the tip of the gas-delivery tube. This can be easily re-dissolved by placing the delivery tube for 1 or 2 minutes in a few drops of chloroform in a test-tube; the solution is then washed into the main extract with a little cyclohexane.

Table I gives the approximate limits of detection for some bases at various stages of the procedure; the limits shown are based on practical experience of the method. The limit

of detection attained depends largely on the skill and experience of the operator and involves factors such as the technique of spraying the chromatograms. For instance, if both sides of a chromatogram are sprayed with the acidified iodoplatinate reagent, it is usually found that the sensitivity is greatly increased. Under these conditions, ammonium ion may develop as a grey spot on the pink background at a low R_F value. It is sound practice initially to carry out some blank extractions in order to ascertain whether or not (*a*) the solvents or paper used make any contribution and (*b*) the ammonia introduced just before development of the chromatogram can be detected.

I acknowledge that this work was made possible by grants from the Victoria Racing Club and the Anti-Cancer Council of Victoria.

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1. Morgan, P. J., *Analyst*, 1959, **84**, 418.
2. Cole, A. R. H., *Rev. Pure Appl. Chem.*, 1954, **4**, 118.
3. Ard, J. S., *Anal. Chem.*, 1953, **25**, 1743.

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A Vanillin - Perchloric Acid Reagent for Detecting Pregnanetriol and Related Compounds on Paper Chromatograms

BY J. D. FEW

(*Department of Chemical Pathology, Charing Cross Hospital Medical School, London, W.C.2*)

The conditions under which a vanillin - perchloric acid reagent can be used for detecting pregnanetriol on paper chromatograms are described. As well as with pregnanetriol, the reagent produces distinctive colours with several other closely related steroids.

Other aromatic aldehydes in conjunction with perchloric acid have been investigated as colour-forming reagents for steroids, and the results are reported.

DURING a study of the urinary steroids excreted in cases of congenital adrenal hyperplasia, the need arose for a simple and sensitive method of detecting pregnanetriol (5β-pregnane-3α,17α,20α-triol) on paper chromatograms. Of the reagents previously used for this purpose, molybdophosphoric and phosphotungstic acids[1] are sensitive and simple to use, but have the disadvantage of reacting with a wide range of steroids to form blue spots only. Antimony trichloride, in either chloroform[2] or nitrobenzene,[3] reacts with fewer steroids and is sensitive to pregnanetriol, but is highly toxic and easily affected by traces of water or alcohols.

Bush[4] described the oxidation of steroidal 17,20-glycols with periodic acid and detection of the generated 17-oxosteroids with alkaline *m*-dinitrobenzene. Although this technique has the requisite sensitivity and is specific for 17,20-glycols, it is neither simple nor rapid to carry out. The use of trichloroacetic acid as a reagent for pregnanetriol was described by de Courcy[5]; this reagent forms distinctive colours visible in the visual and ultra-violet regions with pregnanetriol and a few closely related compounds. Satisfactory development of colour, however, depends on careful control of the heating of the papers, during which process unpleasant fumes are evolved.

Godin[6] described a vanillin - perchloric acid reagent for locating sugar alcohols and ketoses (but not aldoses) on paper chromatograms, and this was later used to locate a wide range of deoxy sugars.[7] Initial experiments in which this reagent was applied to the steroid field showed it to be of value for detecting 17-hydroxy-20-ketones and 17,20-glycols.

Aromatic aldehydes in the presence of sulphuric or orthophosphoric acid have been used for locating a wide range of steroids,[8,9,10,11] but the subject has not apparently been systematically investigated. This paper describes the results of a detailed study of the use of vanillin and other aromatic aldehydes in the presence of perchloric acid for locating steroids on paper chromatograms, with particular reference to the pregnanetriol group.

Experimental

Paper chromatography—

This was carried out with use of the system iso-octane - toluene - methanol - water in the ratio 50:150:160:40 (as recommended by de Courcy[5]) and 80:120:160:40. Whatman No. 1 chromatography paper was used throughout. In exploratory experiments, spots of solutions of steroids were placed on test strips of paper to give a concentration of 5 μg of steroid per sq. cm, and these strips were treated with the colour-forming reagents without chromatographic development.

Colour-forming reagents—

These were prepared in aqueous solution so as to contain 1 per cent. w/v of the aldehyde and 10 per cent. v/v each of perchloric acid and either toluene-*p*-sulphonic or orthophosphoric acid. For aldehydes sparingly soluble in water, up to 20 per cent. v/v of acetone was included in the mixture. A typical reagent solution consisted of 1 g of vanillin, 10 g of toluene-*p*-sulphonic acid and 15 ml of 60 per cent. perchloric acid *plus* water to 100 ml.

Treatment of chromatograms—

The dried papers were dipped in the reagent, excess of solution was removed by blotting, and the papers were dried in a stream of warm air (75° to 85° C) from a laboratory air blower or an electric convection heater; the spots of steroids appeared when the paper was dry. With the reagent containing vanillin, the coloured spots appeared on a yellow background, which rapidly faded. Excessive heating of the paper was avoided, as it led to charring (as did heating in a closed oven at 100° C).

Results

Composition of colour-forming reagent—

In preliminary experiments on a small range of steroids, a 1 per cent. w/v solution of vanillin containing different proportions of perchloric acid was used; it was found that the intensity of colour was dependent on the concentration of acid present. The concentration of 10 per cent. v/v of perchloric acid was chosen because it led to an adequate colour without causing serious damage to the paper. Concentrations of perchloric acid greater than 15 per cent. v/v were found to produce faint grey spots with almost all steroids, thereby decreasing the specificity.

When perchloric acid was replaced by other acids, pregnanetriol produced little colour except when toluene-*p*-sulphonic or orthophosphoric acid was substituted (and even then the colours were much less intense than those formed in presence of perchloric acid). If toluene-*p*-sulphonic or orthophosphoric acid is added to the vanillin - perchloric acid reagent, then the colour formed by pregnanetriol (and several other steroids) is considerably intensified, without the disadvantages associated with an increased concentration of perchloric acid.

Colours produced with pure steroids—

When the mixture of vanillin, toluene-*p*-sulphonic acid and perchloric acid, subsequently referred to as the vanillin - perchloric acid reagent, was used, 5 μg of any of the compounds listed in Table I could be readily detected on paper chromatograms and 2 μg were just detectable; on the test strips, 1 μg per sq. cm was detectable. The colours produced initially with most of these steroids change during about 30 minutes when exposed to air at room temperature and then gradually fade. The paper is attacked by the reagent, but only disintegrates after a few days, by which time most of the spots have faded.

Derivatives of 3,4-dihydroxybenzaldehyde other than vanillin react similarly with this group of steroids; 2,4-dihydroxybenzaldehyde and *p*-hydroxybenzaldehyde also react with the steroids listed in Table I, but the colours formed differ slightly from those produced by vanillin.

As well as with this group of compounds, the vanillin - perchloric acid reagent also reacts with other steroids, although the colours formed are less intense. These steroids, which are listed in Table II, generally produce more intense colours with *p*-hydroxybenzaldehyde than with vanillin; with 2,4-dihydroxybenzaldehyde they react either not at all or only to produce colours very low in intensity. If the concentration of perchloric acid in the reagent

is increased to 15 per cent. v/v, then *p*-hydroxybenzaldehyde with the addition of 10 per cent. v/v of orthophosphoric acid is a suitable reagent for detecting most of the steroids listed in Table II, 10 μg being easily detectable on paper chromatograms. A blue-grey colour is produced by most of these compounds, but the sapogenins appear as yellow spots.

The corticosteroids cortisone, cortisol and cortexone and the saturated 17-oxosteroids not substituted in the 16-position do not react with any of the aldehyde - perchloric acid reagents at concentrations of less than 20 μg per sq. cm on test strips and give mostly negative results at 50 μg per sq. cm. Other steroids not reacting with these reagents include testosterone, 3α-hydroxy-5β-pregnan-20-one, 3α,16α-dihydroxy-5β-pregnan-20-one and lithocholic acid. Deoxycholic acid reacts with *p*-hydroxybenzaldehyde to produce an intense colour, but not with any of the other aldehydes tested.

In many chromatographic systems, solvents having elevated boiling-points are used as stationary phase, and these are often difficult to remove completely from the paper chromatograms. Several of these solvents have been tested for reactivity with the vanillin - perchloric acid reagent by placing spots of a (1 + 1) mixture of solvent and methanol on test strips of paper, dipping the strips into the reagent, drying and heating in the usual way after evaporation of the methanol. 2-Ethoxyethanol (ethyl Cellosolve) produced an intense brown colour immediately after drying; propylene glycol formed a red colour after prolonged heating, and formamide inhibited formation of all colour, including the yellow background. Ethylene glycol and glycerol were inactive.

TABLE I

STEROIDS REACTING TO PRODUCE INTENSE COLOURS

Steroid	Colour produced*
5α-Pregnane-3α,17α,20α-triol	Purple changing to blue
5β-Pregnane-3α,17α,20α-triol	
5β-Pregnane-3α,17α,20β-triol	
5β-Pregnane-3α,16α,20β-triol	Mauve changing to blue
3α,17α-Dihydroxy-5β-pregnan-20-one	Orange changing to colourless†
3α-17α-Dihydroxy-5β-pregnane-11,20-dione	
3α,17α-Dihydroxy-5α-pregnan-20-one	
Pregn-5-ene-3β,17α,20α-triol	Green changing to blue
Pregn-5-ene-3β,17α,20β-triol	
Pregn-5-ene-3β,16α,20β-triol	Grey
3β,16α-Dihydroxypregn-5-en-20-one	Orange changing to brown†
Diosgenin	Yellow

* The changes in colour take place during approximately 30 minutes when the chromatogram is exposed to air at room temperature.

† This change can be reversed by re-heating.

TABLE II

STEROIDS REACTING TO PRODUCE LESS INTENSE COLOURS

Steroid	Colour produced
Cholic acid	Mauve
Hecogenin	Yellow
Tigogenin	
5α-Androstane-3β,17β-diol	Blue
3β,16α-Dihydroxy-5α-androstan-17-one	
Pregnane-3,20-diol (various isomers)	
3α,17α,21-Trihydroxy-5β-pregnan-20-one	Dull purple
3β-Hydroxyandrost-5-en-17-one	Grey-brown
3β-Hydroxypregn-5-en-20-one	
Androst-5-ene-3β,17β-diol	
Pregn-5-ene-3β,20β-diol	

Among the other aldehydes tested, phthalaldehyde (without the addition of orthophosphoric or toluene-*p*-sulphonic acid) was useful, as it produced an intense blue colour with the epimeric pregn-5-ene-3β,17α,20-triols and only a pale brown with pregnanetriol. This reagent also reacted with all the other Δ^5-3β-hydroxy-steroids listed in Tables I and II,

giving a characteristic olive colour that permitted the easy detection of 5 μg of any of these steroids on paper chromatograms. It did not react with any of the other steroids tested.

p-Dimethylaminobenzaldehyde and pyridine-3-aldehyde (also without the addition of orthophosphoric or toluene-*p*-sulphonic acid) appear to be fairly specific for the Δ^5-3β-hydroxyl grouping, but neither of these aldehydes produces such an intense colour as do the other reagents, and, especially with pyridine-3-aldehyde, the spots fade rather rapidly.

Although other aromatic aldehydes, including benzaldehyde, furfural, 2,3-dimethoxybenzaldehyde and anisaldehyde, form colours with some steroids in the presence of perchloric acid, they were not found to be of value in this type of analytical work, owing to lack of sensitivity, often combined with lack of specificity and instability of colour developed. Many aldehydes, including all the mononitro- and monochlorobenzaldehydes, *m*-hydroxybenzaldehyde and various hydroxynitrobenzaldehydes, do not form colours with any of the steroids tested.

Application to extracts of urine—

Extracts of urines hydrolysed with β-glucuronidase were fractionated on columns of alumina by the method used by Stern[12] in the determination of pregnanetriol. The "pregnanediol" and pregnanetriol fractions were submitted to paper chromatography, and the chromatograms were treated with the vanillin - perchloric acid reagent. The "pregnanediol" fraction always showed a number of spots, of which that produced by 3α,17α-dihydroxy-5β-pregnan-20-one was usually prominent. It is only in urine from pregnant subjects that pregnanediol occurs in amounts sufficient to be detected in Stern's "pregnanediol" fraction with this reagent. When the pregnanetriol fraction was examined, pregnanetriol was generally found to be the major compound reacting with vanillin. With urine from several cases of congenital adrenal hyperplasia, pregnanetriol was the only compound reacting with vanillin that was detectable in this fraction. In extracts from normal subjects, and particularly after stimulation with adrenocorticotropic hormone, many other compounds were present besides pregnanetriol. Mostly, these other compounds were not chromogenic with sulphuric acid and hence did not interfere with the determination of pregnanetriol with this reagent.

When paper chromatograms of many extracts of urine are treated with the vanillin - perchloric acid reagent, some of the spots formed do not correspond in position or colour to those produced by any of the available reference compounds. At present, several of these substances are being investigated, although at this stage there is no definite evidence that they are steroids.

Discussion of the technique

The initial aim of this work was to devise a reagent suitable for locating pregnanetriol and related compounds on paper chromatograms. When this aim had been achieved, with the vanillin - perchloric acid reagent, it seemed at least possible that, if other aldehydes were used, this type of reaction could be extended to other groups of steroids. Unfortunately, examination of thirty other aromatic aldehydes has led to the conclusion that this type of reagent has little application outside the pregnanetriol group, except when low sensitivity is acceptable or unavoidable.

Noteworthy exceptions to this conclusion are the sapogenins, which produce intense yellow colours with the vanillin - perchloric acid reagent. Recently, Slack and Mader[13] have utilised a yellow colour formed by diosgenin and 70 per cent. perchloric acid as the basis of a colorimetric determination of diosgenin in the presence of saturated sapogenins (which are not chromogenic with perchloric acid). In contrast to the reaction described by Slack and Mader, the reaction with the vanillin - perchloric acid reagent is given by saturated sapogenins as well as by diosgenin, although the latter compound produces a colour rather more intense than that formed by hecogenin or tigogenin.

At present, it is not possible to explain the mechanism of this colour reaction with any confidence. Experiments are in progress to adapt it for quantitative work, and this should facilitate the elucidation of the mechanism of the reaction.

Several reagents previously used for detecting pregnanetriol on paper have to be applied as a spray, and, during this process or subsequent heating, highly toxic fumes may be evolved. In 2 years' experience with the vanillin - perchloric acid reagent, I have never found it to be in the least objectionable when used as described above.

I am grateful to Professor J. Patterson for his interest in this work.

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The Determination of Aromatic Amino-compounds

By J. W. DANIEL

(*Imperial Chemical Industries Ltd., Industrial Hygiene Research Laboratories, The Frythe, Welwyn, Herts.*

The efficiencies of five coupling reagents used for the colorimetric determination of aromatic amines and amino-compounds have been compared. Although *N*-sulphatoethyl-*m*-toluidine was perfectly satisfactory for reactions involving short periods of coupling, *N'*-diethyl *N*-1-naphthylpropylenediamine is recommended for routine application.

Aromatic amines are of considerable industrial importance, and a health hazard is associated with the manufacture of many of them, *e.g.*, aniline, 2-naphthylamine and the toluidines and xylidines. The control of industrial exposure necessitates frequent analyses of the atmosphere, and a sensitive analytical procedure for determining these compounds has obvious advantages. Such a method should also be applicable to the study of the *in vivo* metabolism of those organic compounds (*e.g.*, nitrobenzene, "azo" food colours and nitrophenols) excreted as the corresponding amines, such as aniline and sulphanilic acid, the determination of which in biological fluids is important in metabolic investigations.

Various procedures have been described for determining amino-compounds.[1] When the amine is present in microgram amounts, the favoured procedure involves diazotisation with nitrous acid and then reaction with a suitable coupling reagent to give a highly coloured azo-compound. The intensity of the colour can then be related to the concentration of the amine by using a photoelectric colorimeter or some other convenient method. Since the sensitivity depends on the coupling component, an assessment has been made of the efficiencies obtained with a variety of coupling agents in the determination of a representative group of amines, *viz.*, aniline, the isomeric chloroanilines, toluidines and xylidines and sulphanilic and metanilic acids. The coupling reagents selected included those that have been widely used, namely, 3-hydroxy-2-naphthoic acid,[2] 2-naphthol-3,6-disulphonic acid (R salt),[3] *N*-sulphatoethyl-*m*-toluidine[3] and *N*-1-naphthylethylenediamine,[4] and a new reagent, *N'*-diethyl *N*-1-naphthylpropylenediamine ("Prolabo," Rhône - Poulenc).

Experimental

The amine (0·100 g) was dissolved in a little dilute hydrochloric acid, and the solution was diluted to 100 ml with water; further dilutions were made from this solution as required.

Diazotisation—

A 1-ml portion of solution, containing 10 μg of amine, was placed in each of a series of glass-stoppered tubes, and to the contents of each tube were then added 1 ml of 2 *N* hydrochloric acid and 1 ml of a 0·25 per cent. solution of sodium nitrite. Stoppers were inserted

in the tubes, and the contents were mixed by inversion. A reagent blank was prepared simultaneously, and the tubes were set aside at room temperature for 15 minutes before proceeding with the coupling stage.

COUPLING—

The various reagents were added to the diazotised amine, and coupling was allowed to proceed until maximum development of colour had been attained. The wavelengths at which the colours absorbed maximally were established in preliminary experiments, and standard graphs were plotted relating optical density of the coloured solution to the concentration of amine present. From the slopes of these graphs, the optical density (10-mm cell) equivalent to 1 μg of amine per ml of final solution was calculated for each amine, and the figure so obtained was used as the basis for comparing the efficiencies of the individual coupling reagents. All optical-density measurements were made with a Unicam SP600 spectrophotometer.

REAGENTS AND TECHNIQUES

2-NAPHTHOL-3,6-DISULPHONIC ACID—

From 15 to 16 g of the disodium salt of the reagent were dissolved, with heating, in 500 ml of water, the solution was made just alkaline to brilliant yellow paper with M sodium carbonate, cooled, filtered and made up to 1 litre with water. To each portion of diazotised amine were added 10 ml of M sodium carbonate and 0·5 ml of the reagent solution, and the volume was adjusted to 25 ml with water.

N-SULPHATOETHYL-*m*-TOLUIDINE—

To each portion of diazotised amine were added 2 ml each of 3 M sodium acetate and a 1 per cent. w/v solution of the reagent. The azo colour was developed by adding 0·5 ml of concentrated hydrochloric acid, and the volume was made up to 10 ml with water. For coupling periods in excess of 60 minutes, 1 ml of a 2·5 per cent. w/v solution of ammonium sulphamate was added before the 3 M sodium acetate.

3-HYDROXY-2-NAPHTHOIC ACID—

To each portion of diazotised amine was added 1 ml of the 2·5 per cent. w/v solution of ammonium sulphamate, and, 1 minute later, 1 ml of a 0·60 per cent. solution of the reagent in M sodium carbonate was added. The solution was transferred to a separating funnel, and 50 per cent. v/v sulphuric acid was added until the solution was just acid to Congo red paper. The solution was successively extracted with five 5-ml portions of chloroform, the extracts were combined in a 25-ml calibrated flask, and this solution was diluted to the mark with chloroform.

N-1-NAPHTHYLETHYLENEDIAMINE AND *N'*-DIETHYL *N*-1-NAPHTHYLPROPYLENEDIAMINE—

Each of these reagents was used in 1 per cent. w/v solution, and the procedure adopted for coupling was one of those described below.

A. To each portion of diazotised amine was added 1 ml of the 2·5 per cent. solution of ammonium sulphamate, and, 1 minute later, 1 ml of the reagent solution was added; the solution was then diluted to 10 ml with water.

B. To each portion of diazotised amine was added 1 ml of the ammonium sulphamate solution, and, 1 minute later, 2 ml of 3 M sodium acetate and 1 ml of reagent solution were added. The azo colour was developed by adding 0·5 ml of concentrated hydrochloric acid, and the solution was diluted to 10 ml with water.

DISCUSSION OF RESULTS

The results of the tests are summarised in Table I, from which it is clear that *N*-1-naphthylethylenediamine, *N'*-diethyl *N*-1-naphthylpropylenediamine and *N*-sulphatoethyl-*m*-toluidine provide the same order of sensitivity and in this respect are much superior to 2-naphthol-3,6-disulphonic acid or 3-hydroxy-2-naphthoic acid. The last-named reagent is also limited in application to those amino-compounds forming a chloroform-soluble azo-derivative. It is, however, the only reagent adequate for determining both 2,6- and 2,5-xylidine, which couple slowly with the other reagents; maximum development of colour with the xylidines is

TABLE I

DETAILS OF COLOURS PRODUCED WITH VARIOUS AMINES

Amine	Colour formed with *N*-1-naphthylethylenediamine			Colour formed with *N'*-diethyl *N*-1-naphthylpropylenediamine			Colour formed with *N*-sulphatoethyl-*m*-toluidine		
	Optical density × 10^3	Wavelength of maximum absorption, mμ	Colour-development period, minutes	Optical density × 10^3	Wavelength of maximum absorption, mμ	Colour-development period, minutes	Optical density × 10^3	Wavelength of maximum absorption, mμ	Colour-development period, minutes
Aniline	44*	545	15	45*	540	15	32	500	10
o-Toluidine	25*	540	30	34*	535	30	13	505	60
m-Toluidine	33*	545	15	34*	540	15	27	505	10
p-Toluidine	38*	545	30	43*	545	30	20	505	10
2,6-Xylidine	<1*	530	60	1*	545	60	<1	505	60
2,4-Xylidine	13*	545	120	18*	540	120	18	505	180
2,3-Xylidine	4*	535	60	8*	530	60	2	515	60
3,4-Xylidine	22*	550	30	22*	555	30	40	515	45
2,5-Xylidine	5*	535	60	8*	535	60	11	500	120
o-Chloroaniline	30†	540	15	30†	535	15	30	500	60
m-Chloroaniline	35†	540	15	35†	535	15	33	500	10
p-Chloroaniline	36†	545	15	37†	540	15	33	505	10
Sulphanilic acid	27†	535	15	27†	530	15	27	505	15
Metanilic acid	25†	535	15	24†	535	15	—	—	—

Amine	Colour formed with 3-hydroxy-2-naphthoic acid			Colour formed with 2-naphthol-3,6-disulphonic acid		
	Optical density × 10^3	Wavelength of maximum absorption, mμ	Colour-development period, minutes	Optical density × 10^3	Wavelength of maximum absorption, mμ	Colour-development period, minutes
Aniline	32	500	30	6	485	30
o-Toluidine	2	505	30	11	485	60
m-Toluidine	5	505	30	9	485	30
p-Toluidine	9	505	30	9	495	30
2,6-Xylidine	<1	500	60	4	485	60
2,4-Xylidine	5	515	60	7	495	60
2,3-Xylidine	1	510	60	3	485	60
3,4-Xylidine	7	515	30	4	510	30
2,5-Xylidine	3	505	60	7	485	120
o-Chloroaniline	5	500	30	4	485	15
m-Chloroaniline	6	500	30	4	485	15
p-Chloroaniline	6	500	30	5	485	15

* Coupling carried out by procedure B (p. 641). † Coupling carried out by procedure A (p. 641).

achieved more quickly with this reagent and with 2-naphthol-3,6-disulphonic acid, although the sensitivity obtained is low. The time taken to attain maximum development of colour is approximately the same for *N*-sulphatoethyl-*m*-toluidine, *N*-1-naphthylethylenediamine and *N'*-diethyl *N*-1-naphthylpropylenediamine. The effect of the buffering action of sodium acetate (pH 9) on coupling is pronounced for those amines not containing an electro-negative substituent, *e.g.*, aniline and the toluidines and xylidines. The effect of this buffering action on the rate of coupling was found to be greater with *N'*-diethyl *N*-1-naphthylpropylenediamine than with *N*-1-naphthylethylenediamine. *N*-Sulphatoethyl-*m*-toluidine does not couple in absence of sodium acetate.

The magnitude of the reagent blank is important, as low values permit greater analytical sensitivity at low concentrations of amines. Experience in this laboratory with *N*-1-napthylethylenediamine has demonstrated wide variation in the quality of different batches of this reagent, with subsequent high blank values. The single batch of *N'*-diethyl *N*-1-naphthylpropylenediamine so far examined gave a blank value much lower than that hitherto obtained with *N*-1-naphthylethylenediamine. For reactions involving short periods of coupling, *N*-sulphatoethyl-*m*-toluidine is a satisfactory reagent, but, for the reasons outlined, *N'*-diethyl *N*-1-naphthylpropylenediamine has been adopted in this laboratory as the most satisfactory reagent for routine use.

I thank Mr. G. R. Norton for assistance during part of this work.

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The Identification of Substances of Low Volatility by Pyrolysis/Gas-Liquid Chromatography*

By G. C. Hewitt and B. T. Whitham

(*"Shell" Research Ltd., Thornton Research Centre, P.O. Box No. 1, Chester*)

The use of pyrolytic degradation for the identification of substances of low volatility and also for more fundamental studies of the break-down mechanisms of polymers is reviewed. Particular emphasis is placed on the advantages of gas - liquid chromatography for the analysis and identification of products of pyrolysis.

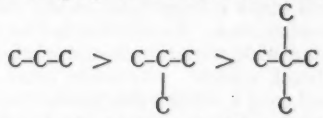
A description is given of a glass pyrolysis unit that can be connected to an analytical gas - liquid chromatographic column. Solid and liquid samples can be readily introduced into the heated pyrolysis zone of this unit, and typical chromatograms of polymer-degradation products are shown. It is concluded that polymers can be readily classified according to type from their pyrolysis chromatograms and that, often, individual polymers among a given type can also be classified.

The analyst is often presented with the problem of identifying the polymers and other complex substances that occur in many natural and synthetic products. In most instances, degradation processes of some type have to be used in an attempt to isolate discrete fragments of these substances, which can be identified by their chemical and physical properties. The evidence obtained by characterisation of these fragments, together with a knowledge of the properties of the original material, leads to the identification of the complex substance.

* Presented at the joint meeting of the North of England Section and the Physical Methods Group of the Society on Thursday, September 29th, 1960.

* Coupling carried out by procedure B (p. 641). † Coupling carried out by procedure A (p. 641).

In the study of polymeric substances, pyrolytic degradation has been extensively used. As long ago as 1862, Williams[1] used pyrolysis to isolate the basic isoprene unit from natural rubber, and several publications have described the use of pyrolysis as a method of obtaining information about the structures of substances. The mechanism of thermal degradation of polymers has been studied by Madorsky and Straus,[2] who have shown that the types and relative amounts of the products of pyrolysis are functions of the molecular structure and the kind and frequency of side-groups. These workers have shown how, for instance, the thermal stability and break-down products obtained on pyrolysis can be related to the strengths of the C–C bonds in the polymer chain, *i.e.*, secondary > tertiary > quaternary.



In recent years, indirect "finger-printing" methods have been used in conjunction with pyrolytic methods. Infra-red[3] and mass spectrometry,[4,5] for example, have been used to "finger-print" the total products of pyrolysis from various polymers. This type of approach often involves compilation of a library of reference spectra obtained from pyrolysis of substances of known structure, and the identity of an unknown is obtained by referring to these spectra. The approach is generally successful, as the products of pyrolysis obtained under given conditions are specific for each type of substance. With some substances, such as linear homopolymers, direct identification is possible, as the conditions of pyrolysis can be selected so that the monomer is obtained.

The usefulness of pyrolytic degradation has been greatly enhanced since the advent of gas - liquid chromatography,[6] which is basically a rapid method for separating small amounts of volatile substances. In this technique, the substances are carried through a small packed column by means of a stream of inert gas, and separation efficiencies of many thousands of theoretical plates can be achieved. The column packing invariably consists of a powdered porous solid phase coated with a non-volatile liquid. Generally, separation depends largely on the relative volatilities of the substances, but considerable variations in the order of separation of different types of substances can be obtained with different non-volatile liquid phases. In recent years,[7,8] the range of gas - liquid chromatography has been extended to the separation of substances having boiling-points as high as 550° C by using columns operated at temperatures up to 300° C. Further extension of the technique to substances even less volatile is limited by lack of suitable stationary phases and by the possibility of decomposing the substances at the high column-operating temperatures required. A combination of pyrolysis with gas - liquid chromatography offers an alternative approach to the analysis of substances having such low volatility. The important features of gas - liquid chromatography that make it an excellent analytical technique for use in conjunction with pyrolytic degradation are as follows: (*i*) the products of pyrolysis are rapidly separated, (*ii*) the separations can easily be recorded automatically, (*iii*) only a few milligrams of sample are required, (*iv*) the products of pyrolysis can be identified by the time each constituent takes to pass through the chromatographic column (the retention time), (*v*) further identification of the products of pyrolysis can be obtained by collecting fractions at the outlet from the column, (*vi*) the chromatogram of the products of pyrolysis of a substance is in itself a "finger-print" and can be used in a similar manner to infra-red spectra for identification purposes and (*vii*) the pyrolysis unit can be connected directly to the inlet of the chromatographic column (this is particularly important, as all the products of pyrolysis can be swept into the column by the carrier gas as soon as they are formed; there is little chance of re-combination of the primary products of pyrolysis with this arrangement).

Applications of the technique

Identification of polymers—

The earliest work on pyrolysis combined with gas - liquid chromatography was carried out by subjecting the sample to pyrolysis, collecting the products in a cold trap and transferring them to the chromatographic column for analysis. Davison, Slaney and Wragg[9] applied this principle to natural rubber, copolymers of butadiene with acrylonitrile or with styrene, poly(methyl acrylate), polyisobutene, poly(vinyl acetate) and poly(ethyl acrylate).

Pyrolysis was carried out at 650° C in a stream of nitrogen at atmospheric pressure, and the products were collected and then examined by gas - liquid chromatography on a column containing dinonyl phthalate as liquid phase and operated at 111° C. "Finger-print" chromatograms were obtained for those products of pyrolysis sufficiently volatile at the latter temperature.

Subsequently, Haslam and Jeffs[10] de-polymerised copolymers of methyl methacrylate with styrene, methyl acrylate and ethyl methacrylate. The de-polymerisation was carried out at 350° C *in vacuo*, under which conditions the monomers were obtained, and these were identified from their retention times.

Direct coupling of the pyrolysis unit with the gas - liquid chromatographic column was first described by Radell and Strutz,[11] who subjected acrylate and methacrylate polymers to pyrolysis by immersing a metal loop containing the sample (about 5 mg) in a bath of Wood's alloy at 500° C for 30 seconds. The products of pyrolysis were swept by the carrier gas directly into a 2-metre column containing di-n-decyl phthalate at 100° C; chromatograms

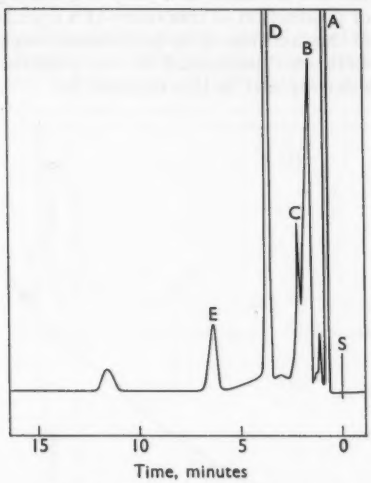


Fig. 1. Pyrolysis chromatogram obtained by Radell and Strutz[11] for poly-(methyl acrylate): S, sample introduction; A, air; B, methanol; C, ethanol; D, methyl acrylate; E, methyl methacrylate. (Reprinted from *Analytical Chemistry*, 1959, **31**, 1891)

characteristic of each polymer were obtained. Under these conditions, acrylate polymers were degraded to mixtures of the monomer, short-chain alcohols and various fragments (see Fig. 1); methyl, ethyl and n-butyl methacrylate polymers were degraded primarily to their monomers. The analysis of polymethacrylates has also been carried out by decomposing the polymer coated on a heated filament at the inlet of the gas - liquid chromatographic column and identifying the resulting monomers by their retention volumes.[12]

COMPOSITION OF COPOLYMERS—

The compositions of copolymers have been determined by coating or placing the sample on a filament that can be rapidly brought to any specified temperature for a brief period. Samples are degraded at a particular temperature, and the composition of the copolymer is determined by measuring certain peaks on the chromatogram characteristic of the individual monomers. In this way, vinyl chloride - vinyl acetate copolymers have been analysed by means of the peaks for hydrogen chloride and acetic acid.[13] Similarly, the compositions of copolymers of methyl methacrylate with methyl acrylate have been determined by comparison of the heights of the peaks recorded for methyl methacrylate monomer and for

methanol *plus* methyl acrylate monomer with those obtained by pyrolysis of samples of known composition.[14] Fig. 2 shows a typical calibration curve obtained in this quantitative work, and it is claimed that the components of these copolymers can be determined with a precision of $\pm 0{\cdot}5$ per cent.

BREAK-DOWN MECHANISMS OF HIGH POLYMERS—

Lehrle and Robb[13,15] have also used the technique with the heated wire spiral for more fundamental studies of the break-down mechanisms of high polymers by recording chromatograms of the products of degradation from the same sample of polymer maintained for a few seconds at each of a series of temperatures up to 1000° C. Information about the importance of the various degradation processes throughout the range of temperatures can be obtained by measuring the relative proportions of the products of degradation at each temperature. In this way, it has been shown that the principal process in the degradation of poly(vinyl acetate) is the production of acetic acid at intermediate temperatures and that the polyacetylene skeleton formed is stable and only degraded at the highest temperatures.

By measuring the rate of production of monomer at a specified temperature, the energies of activation and the rates of degradation of de-polymerisation processes can be determined. It is possible to differentiate between random and block copolymers by means of the differences in their rates of degradation determined in this manner.[15]

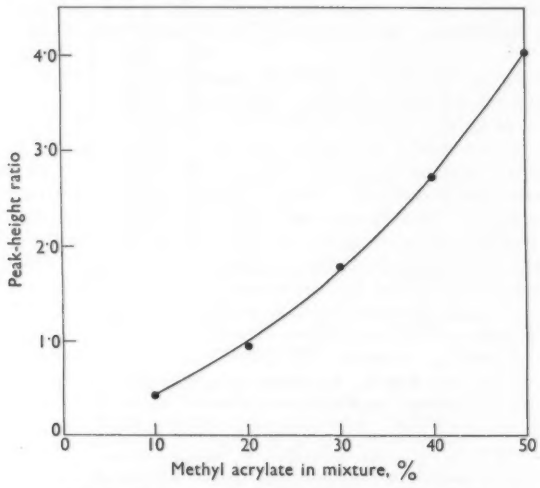


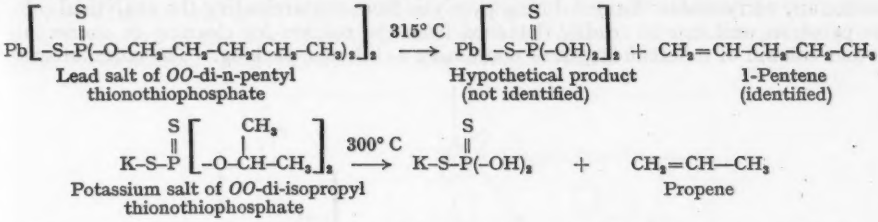
Fig. 2. Curve plotted by Strassburger, Brauer, Tryon and Forziati[14] for the peak-height ratios of methanol *plus* methyl acrylate to methyl methacrylate in a series of polymer mixtures. (Reprinted from *Analytical Chemistry*, 1960, **32**, 456)

IDENTIFICATION OF COMPOUNDS OTHER THAN POLYMERS—

Janák[16,17] has shown that it is possible to identify various barbiturates, plant oils (*e.g.*, olive, linseed, castor and coconut oils), amino acids and alkaloids by pyrolysis of a sample on a heated filament in the inlet gas stream of a gas - liquid chromatographic column. Unknown substances were generally identified by reference to chromatograms recorded after pyrolysis of substances of known composition. Janák gave an example to illustrate the possibilities of this analytical technique. A child had unfortuntely consumed tablets of an unknown nature, and examination of an ether extract of the child's urine by pyrolysis/gas - liquid chromatography yielded a chromatogram almost identical with that of veronal (diethylbarbituric acid); the chromatograms obtained are shown in Fig. 3.

The structures of certain compounds added to petroleum, such as organic phosphates and thiophosphates, are difficult to characterise by normal methods, owing to the chemical

reactivity of these substances, their resistance to hydrolysis and their poor thermal stability. Legate and Burnham[18] have described how gas - liquid chromatography of the products of pyrolysis can be successfully used for identifying the organic radicals in such compounds. Their pyrolysis unit, which was attached directly to the chromatographic column, consisted of a stainless-steel tube packed with glass-wool and heated in a furnace; two examples of the type of pyrolytic reaction they obtained are shown below.



Since pyrolysis/gas - liquid chromatography alone cannot distinguish between a n-propyl and an isopropyl group, it would be necessary to obtain the infra-red spectrum of the C_3-thionothiophosphate to prove the presence of the iso-configuration.

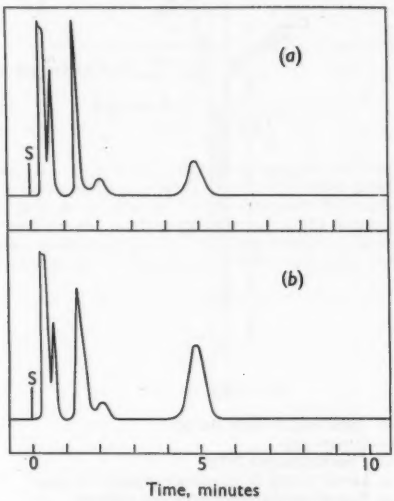


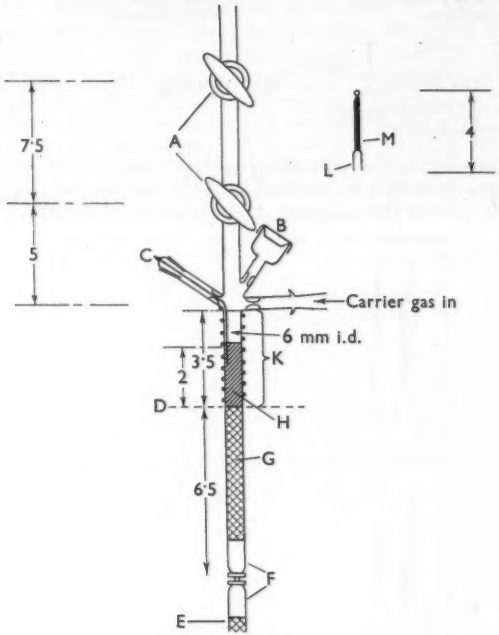
Fig. 3. Identification of diethylbarbituric acid (veronal) in urine. Chromatograms obtained by Janák[17] for (*a*) veronal standard and (*b*) extract from urine: S, sample introduction. (Reproduced by permission of the author and Butterworths Publications Ltd.)

Variations of the technique have recently been described,[19] in which the pyrolysis tube is replaced by a small heated reaction chamber containing palladium on activated alumina. Sulphur compounds introduced into this reaction chamber were de-sulphurised, and the hydrocarbon fragments were identified from their retention volumes or by mass-spectrometric examination of the collected fractions. It was possible to arrive at the identity of the original sulphur compound from the identities of the fragments.

Description of apparatus

So far, the main emphasis of our work has been on the pyrolysis of polymers as a means of identification. The pyrolyses have been carried out in the glass unit shown in Fig. 4. This unit is coupled directly to the chromatographic column, and both liquid and solid

samples can be conveniently handled. Solid samples are introduced by means of a capillary tube containing an iron core; after pyrolysis of the sample has been completed, the tube can be withdrawn by means of a magnet. A hypodermic syringe is used to introduce liquid samples via the rubber serum cap. The pyrolysis chamber is heated electrically by means of windings on the outside of the tube, and the temperature inside the unit is measured by a thermocouple. The few centimetres of column packing before the chromatographic column prevent any tarry matter formed during pyrolysis from contaminating the analytical column. The pyrolysis unit can be readily detached from the column for cleaning or connecting to another column of different length or containing a different packing. The chromatographic



A = Taps (extra wide bore)
B = Serum cap
C = Thermocouple inlet
D = Level of top of chromatography furnace
E = Top of gas-chromatographic column
F = Glass-to-metal seals
G = Layer of stationary phase
H = Lightly packed layer of glass-wool
K = Heated pyrolysis zone
L = Capillary tube for introducing solid samples
M = Iron "slug"

Fig. 4. Demountable pyrolysis unit and sample-introduction system (all dimensions in centimetres)

column was generally packed with 52- to 60-mesh Sil-O-Cel firebrick impregnated with 26 per cent. w/w of silicone grease (E301), and two columns of lengths 6 and 12 feet have been used. The detector was a thermal-conductivity cell, and the outlet was connected to a manifold and trap system[20] for the recovery of fractions for further examination. The carrier gas was nitrogen, and at the rates of flow normally used the residence time of vaporised substances in the pyrolysis chamber would be about 1 second.

Results for polymers

We have used the apparatus described above for examining several reference polymers. The pyrolysis chamber was generally maintained at 378° C, and substances that did not

decompose at this temperature were successfully decomposed at 435° C. The weight of sample used was approximately 10 mg.

In general, all the substances examined have given pyrolysis chromatograms sufficiently characteristic for identification purposes; for some substances, such as polyisobutylene and polystyrene, the monomer was the only product (see Fig. 5). The methacrylate polymers studied were degraded down to the olefin corresponding to the alcohol originally forming the methacrylate ester. The chromatogram from a C_{18}, C_{16}, C_{14}, C_{12} and C_4 methacrylate copolymer is shown in Fig. 6; this copolymer had been previously analysed by hydrolysing and then recovery of the alcohol constituents for identification. The direct pyrolytic technique is a considerably shorter method of identifying these alcohols.

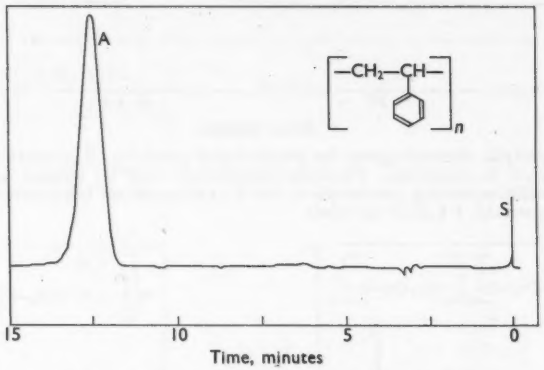


Fig. 5. Pyrolysis chromatogram for polystyrene: S, sample introduction; A, styrene. Pyrolysis temperature, 378° C; 6-foot column of 52- to 60-mesh firebrick impregnated with 25 per cent w/w of silicone E301 grease; column-operating temperature 105° C; katharometer temperature, 60° C; carrier gas (nitrogen) flow rate, 2·9 litres per hour

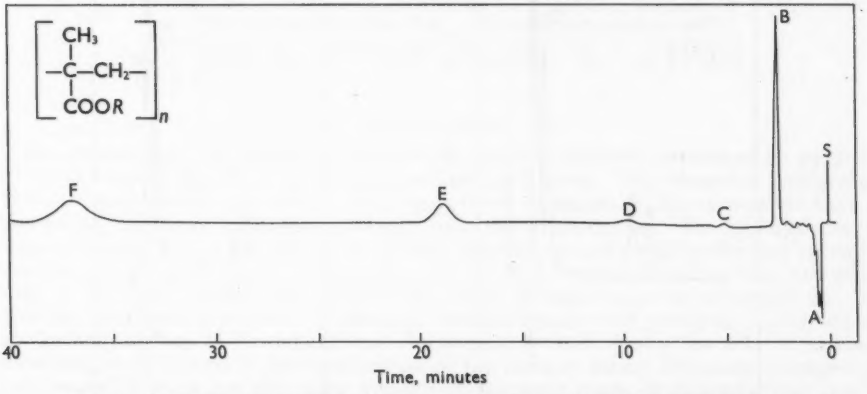


Fig. 6. Pyrolysis chromatogram for C_{18}, C_{16}, C_{14}, C_{12} and C_4 methacrylate copolymer: S, sample introduction; A, butene; B, n-heptane (solvent); C, dodecene; D, tetradecene, E, hexadecene; F, octadecene. Pyrolysis temperature, 438° C; column as for Fig. 5; column-operating temperature, 210° C; katharometer temperature, 160° C; carrier gas (nitrogen) flow rate, 1·6 litres per hour

At 378° C, acrylate polymers were found to give a certain amount of monomer, together with the alcohol and corresponding olefin originally forming the acrylate ester. The chromatogram for poly(n-butyl acrylate) is shown in Fig. 7 (compare with the results of Radell and Strutz in Fig. 1).

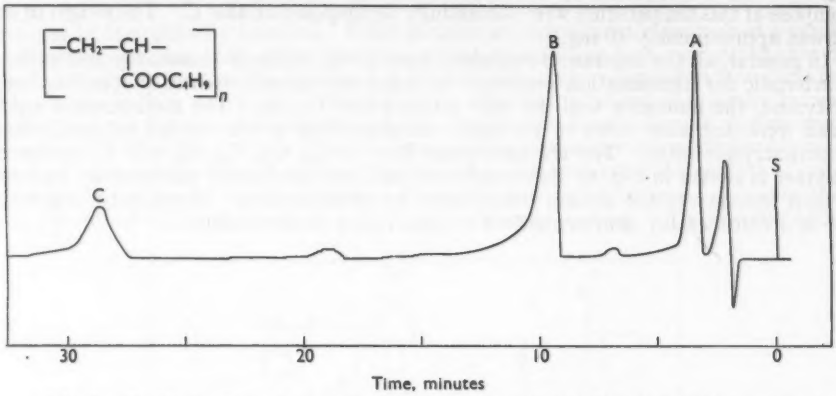


Fig. 7. Pyrolysis chromatogram for poly(n-butyl acrylate): S, sample introduction; A, butene; B, butanol; C, monomer. Pyrolysis temperature, 378° C; column as for Fig. 5, but 12 feet long; column-operating temperature, 150° C; katharometer temperature, 60° C; carrier gas (nitrogen) flow rate, 1·4 litres per hour

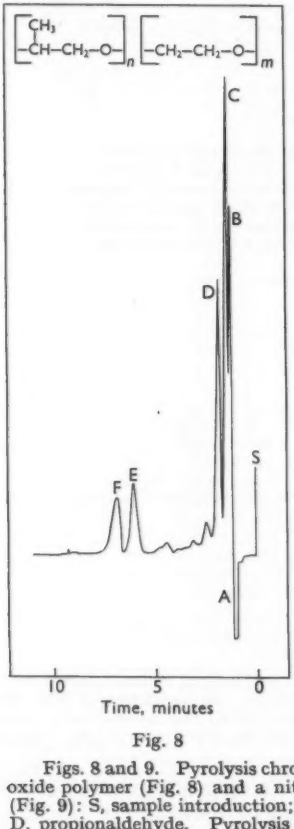


Fig. 8

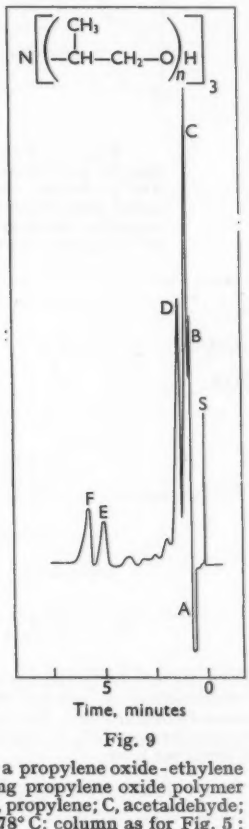


Fig. 9

Figs. 8 and 9. Pyrolysis chromatograms for a propylene oxide-ethylene oxide polymer (Fig. 8) and a nitrogen-containing propylene oxide polymer (Fig. 9): S, sample introduction; A, ethylene; B, propylene; C, acetaldehyde; D, propionaldehyde. Pyrolysis temperature 378° C; column as for Fig. 5; column-operating temperature, 102° C; katharometer temperature, 60° C; carrier gas (nitrogen) flow rate, 2·9 litres per hour

A number of propylene oxide - ethylene oxide polymers have been subjected to pyrolysis; these also gave distinctive chromatograms, and an example is shown in Fig. 8. A nitrogen-containing polymer of this type gave a closely similar chromatogram (see Fig. 9), but differences between the relative proportions of the various peaks were sufficient to distinguish it from the propylene oxide - ethylene oxide polymer.

Poly(vinyl acetate) is another type of polymer examined; for this, it was necessary to use the higher temperature of pyrolysis to obtain degradation. The chromatogram obtained (see Fig. 10) showed one "tailing" peak, indicating a polar compound (probably acetic acid).

Our results confirm that polymers can be readily classified according to type from their pyrolysis chromatograms, and, often, individual polymers among a given type can also be identified.

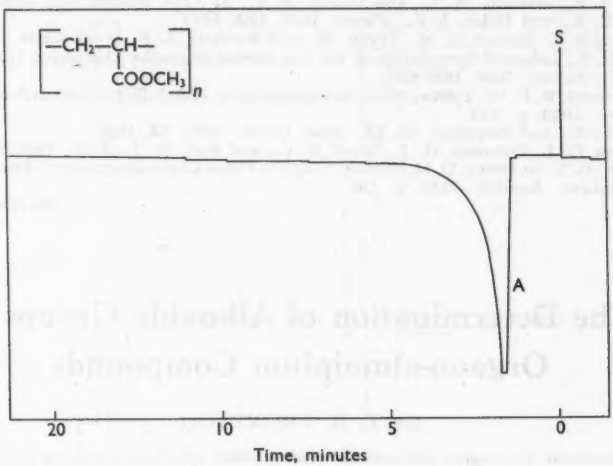


Fig. 10. Pyrolysis chromatogram for poly(vinyl acetate): S, sample introduction; A, acetic acid. Pyrolysis temperature 435° C; column as for Fig. 7; column-operating temperature, 53° C; katharometer temperature, 50° C; carrier gas (nitrogen) flow rate, 2·1 litres per hour

Conclusions

The requirements for the successful identification of unknown substances by pyrolysis/gas - liquid chromatography can be summarised as follows. The elemental composition of the substance should be known. It is important to ensure as far as possible that the substance is free from inhibitors, solvents and other impurities. The pyrolysis of the substance should then be studied at two or three temperatures for which reference chromatograms are available. The chromatographic column should be about 10 feet long and should contain a stationary phase that can be used over a wide range of temperatures. This will ensure that good separation of the most volatile products of pyrolysis is achieved and that the temperature of the column can be increased to elute any less volatile products. The technique of increasing the temperature of the column during separation (temperature programming) is ideal for this work because of the wide range of products that may be obtained. If the resulting chromatograms cannot be matched with any reference chromatograms, the fragments obtained must be identified by means of their retention volumes or spectroscopic examination of recovered fractions, or both.

Gas - liquid chromatography has become so widely used in recent years that any extension of its range of application is greatly welcomed. The rapid identification of substances having low volatility and thermally unstable materials is now possible by the combination of pyrolysis with gas - liquid chromatography, and this technique has clearly become another powerful tool at the disposal of the analyst. Numerous applications in research laboratories can be expected in the future.

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The Determination of Alkoxide Groups in Organo-aluminium Compounds

BY T. R. CROMPTON

(*Analytical Department, Carrington Research Laboratory, Shell Chemical Company Limited, Carrington, Manchester*)

Various organo-aluminium compounds of low molecular weight are extensively used as co-catalysts with titanium compounds in processes for the manufacture of polyethylene and polypropylene by the low-pressure route. A method is described for determining alkoxide groups in these compounds; it is applicable to the determination of alkoxide groups over a wide range of concentrations and is suitable for determining alkoxide-group contents as low as 0·1 per cent.

ALKYLALUMINIUM compounds, such as triethylaluminium and diethylaluminium chloride, usually contain a small amount of alkoxide impurity, owing to the reaction of alkyl groups with traces of atmospheric oxygen that may enter the storage vessel during manufacture of the materials. Alkoxide is formed in accordance with the equation—

$$2AlR_3 + O_2 \longrightarrow 2AlR_2(OR)$$

and a precise method for determining the alkoxide present as impurity is necessary. Alkylaluminium compounds containing one or more alkoxide groups per molecule also have catalytic properties, and a method for determining the alkoxide contents of such compounds is also required.

Alkoxide groups bound to carbon are usually determined by various modifications of the Zeisel method based on reaction with constant-boiling hydriodic acid; the alkyl iodide and olefin produced in the reaction are then determined. A modification of this procedure has also been used to determine alkoxide groups in organo-silicon compounds,[1] but this method is not applicable to organo-aluminium compounds.

The reaction of dilute aqueous acids with aluminium trialkoxides produces the corresponding alcohols in quantitative yield. However, this method of decomposing the sample

could not satisfactorily be applied to alkylaluminium compounds or to their solutions in hydrocarbons because of the highly exothermic nature of the reactions involved; recoveries of the alcohols were variable, even when the reaction mixture was cooled with ice during decomposition. It was found, however, that even the most reactive organo-aluminium compounds could be smoothly decomposed, without loss of the alcohol, when a dilute solution of glacial acetic acid dissolved in toluene was slowly added to the diluted sample maintained at a temperature of −60° C under an atmosphere of nitrogen in a specially constructed reaction vessel. The subsequent addition of an aqueous solution of sodium hydroxide to this mixture dissolved the precipitated aluminium salts and extracted the alcohol into the aqueous phase, from which it could be isolated from electrolytes by steam-distillation and then determined colorimetrically with ammonium ceric nitrate.[2,3]

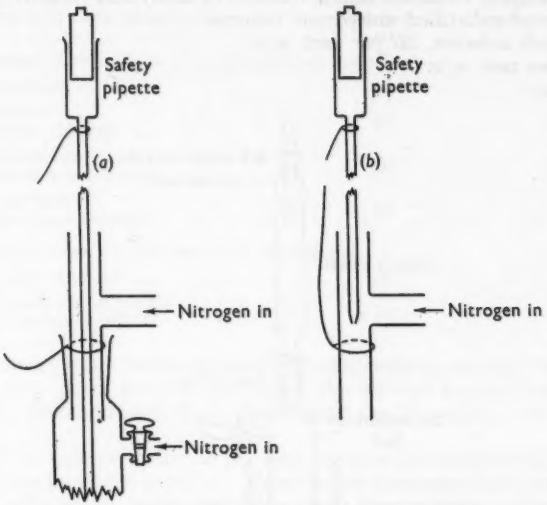

Fig. 1. Diagram showing procedure for sampling organo-aluminium compounds: (*a*) withdrawal or delivery of sample; (*b*) transfer of sample

Neat organo-aluminium compounds containing alkyl groups react explosively on exposure to air and moisture, and contact with these substances must be avoided at all times. Further, as already mentioned, oxygen rapidly converts aluminium-bound alkyl groups to alkoxide groups. Even momentary contact of the neat organo-aluminium compounds or their dilute solutions in hydrocarbons with oxygen during the determination will therefore result in high figures for alkoxide. For these reasons, it is essential to prevent any contamination of the sample by oxygen or moisture during the determination, and this is achieved by maintaining a protective atmosphere of dry oxygen-free nitrogen or another inert gas over the organo-aluminium compound contained in a suitable apparatus. Contamination of the sample is likely to occur at the tip of the pipette during transfer operations, and this is avoided by surrounding the tip with a glass T-piece having a nitrogen inlet on the side-arm, as shown in Fig. 1.

Description of Apparatus

Transfer of organo-aluminium compounds from the sample vessel to the decomposition apparatus is effected by means of dry nitrogen-filled safety pipettes. The sample is decomposed with an acetic acid reagent solution in the apparatus shown in Fig. 2. The decomposition reagents are contained in 100-ml separating funnels, and the cone at the outlet of each separating funnel terminates in a jet (1 mm bore) that serves to control the rate of delivery of reagent to the sample. The decomposition flask is 25 cm tall and has an internal diameter of 4 cm; it stands in a flat-based Dewar flask (10 cm in diameter), which, in turn, stands on a magnetic stirrer.

Isolation of the alcohol from the aqueous extract of the sample is conveniently achieved by using the steam-distillation apparatus recommended by Vogel,[4] which should be so constructed that the volume of the inner sample tube is approximately 150 ml.

METHOD

REAGENTS—

Purified nitrogen—Use purified nitrogen when handling samples and as a "blanket" gas during decomposition of a sample. "Oxygen-free" nitrogen (containing less than 25 p.p.m. of oxygen) from a cylinder is suitable. Dry the nitrogen by passing it through a tower packed with Linde molecular sieve (type 4A).

Toluene—Re-distil, and dry over Linde molecular sieve (type 4A).

Decomposition reagent solution—Mix 3 volumes of analytical-reagent grade glacial acetic acid and 7 volumes of redistilled anhydrous toluene.

Sodium hydroxide solution, 25 per cent. w/v.

Acetic acid, 3 per cent. w/v.

Sodium chloride.

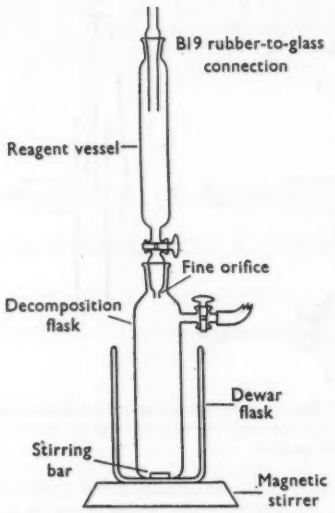

Fig. 2. Apparatus for decomposition of sample

PROCEDURE—

For organo-aluminium compounds (density approximately 0·8) containing up to 5 per cent. by weight of alkoxide groups, use exactly 5 ml of sample; if the compound contains more than 5 per cent. of alkoxide groups, decompose a correspondingly smaller sample. Accurately determine the specific gravity of a separate portion of the sample, and hence calculate the weight of sample used for decomposition. To determine the specific gravity of the compound, deliver a 1-ml sample by means of a dry nitrogen-filled safety pipette into a tared nitrogen-filled weighing flask containing 10 ml of dry toluene, insert a stopper in the flask, and re-weigh.

By pipette, place 20 ml of toluene in a nitrogen-purged dry decomposition flask containing the bar of a magnetic stirrer. Transfer a suitable volume of the sample to the decomposition flask by means of a nitrogen-filled safety pipette equipped with a nitrogen-purged T-piece (see Fig. 1).

Connect a rubber bulb to an oven-dried reagent vessel by means of a suitable rubber-to-glass adapter, and draw in decomposition reagent solution through the orifice until it reaches the level of the stopcock. Close the stopcock, and transfer to the vessel proper the volume of reagent solution necessary to decompose the sample (see Table I); a controlled

delivery of reagent is thereby achieved. Connect the filled reagent vessel to the nitrogen-purged decomposition flask, place solid carbon dioxide and isopropyl alcohol in the Dewar flask to cool to −60° C, and switch on the magnetic stirrer. Replace the nitrogen line by

TABLE I

AMOUNTS OF REAGENTS NEEDED FOR DECOMPOSING VARIOUS ORGANO-ALUMINIUM COMPOUNDS

The amounts shown in this Table are for approximately 5-ml samples

Compound to be decomposed	Volume of decomposition reagent solution needed, ml	Volume of 25 per cent. sodium hydroxide solution needed, ml
Determination of traces of alkoxide in trialkylaluminium compounds—		
Trimethylaluminium	40	50
Triethylaluminium Diethylaluminium chloride	25	30
Tri-n-propylaluminium Di-n-propylaluminium chloride	20	25
Tri-isobutylaluminium Di-isobutylaluminium chloride	15	20
Determination of alkoxide in dialkylaluminium alkoxides—		
Diethylaluminium ethoxide	20	25
Di-n-propylaluminium propoxide Di-isobutylaluminium butoxide	15	20

a vent line to carry away gases produced during decomposition, and add the reagent solution at a rate (about 1 drop per 2 seconds) such that the solvent vapour does not travel more than half-way up the decomposition flask; the reagent can be added more rapidly as decomposition proceeds.

Transfer a suitable volume of the 25 per cent. sodium hydroxide solution to the reagent vessel; this reagent can be added rapidly. Remove the decomposition flask from the Dewar flask, and rinse it with water until it attains room temperature. Transfer its contents, together with aqueous rinsings (about 20 ml), to a separating funnel, and run the lower aqueous layer into a 150-ml calibrated flask. Extract the toluene layer successively with two 20-ml portions of 3 per cent. acetic acid and two 20-ml portions of distilled water. Add the extracts to the contents of the calibrated flask, and dilute to the mark; this is solution A.

Glacial acetic acid sometimes contains a small amount of ethanol as impurity, and this interferes with the determination of alkoxide groups. It is usually necessary, therefore, to carry out a blank determination in order to correct for the presence of this impurity. Determine the blank value exactly as described above, but omit the addition of the organo-aluminium compound; the solution so prepared is solution B.

Transfer 50 ml of solution A and 5 g of sodium chloride to the steam-distillation apparatus, and collect 100 ml of steam-distillate; treat solution B in the same way. The two distillates are suitable for the colorimetric determination of the alcohol with ammonium ceric nitrate solution. Procedures for measuring the orange-red colours produced when this reagent is added to solutions of alcohols have been described; a Spekker absorptiometer with a suitable filter[2] or a Unicam SP600 spectrophotometer at 486 mμ[3] can be used. With either instrument, place solution A in the sample cell and solution B in the comparison cell. The effect on the determination of ethanol present as impurity in the acetic acid is thereby compensated for. Determine the amount of alcohol, originating from the alkoxide, present in solution A by reference to a suitable calibration graph. Calculate the percentage of alkoxide groups in the sample from the expression—

$$\frac{W \times (A - 1{\cdot}008) \times 100}{G \times A}$$

in which W is the weight of alcohol produced (in grams) by the decomposition of G grams of the organo-aluminium compound and A is the molecular weight of the alcohol.

Discussion of results

A sample of aluminium tri-isopropoxide was prepared by reaction of isopropyl alcohol with aluminium (mercuric chloride as catalyst) and was shown by determination of aluminium to have a purity of at least 99 per cent. Contents of aluminium and alkoxide found in this preparation are compared with the theoretical values in Table II. Values for both constituents are close to the theoretical, *i.e.*, the proposed method can be used for determining alkoxide in a comparatively unreactive organo-aluminium compound.

Table II

Results for a sample of aluminium tri-isopropoxide

Constituent determined	Theoretical content, % w/w	Content found, % w/w
Aluminium	13·2	13·1
Isopropoxide	86·8	86·2
Total	100·0	99·3

The determination of alkoxide in highly reactive alkylaluminium and alkylaluminium alkoxide compounds is more susceptible to error, however, because these compounds react exothermically with acetic acid, with consequent risk of loss of alcohol. Further, accidental contamination of alkyl groups by oxygen during decomposition of the sample would cause high results for alkoxide.

Pure samples of these compounds of known alkoxide content were not available, and the accuracy of the proposed procedure could therefore not be checked directly. An indirect check, however, was possible, and Table III shows the results of duplicate determinations of alkoxide in several highly reactive organo-aluminium compounds; a different weight of sample was used in each duplicate determination. It can be seen from these results that

Table III

Alkoxide contents found in various samples

Sample	Weight of sample taken, g	Weight of alcohol isolated, mg	Alkoxide content, %
Triethylaluminium (neat)	2·463	30·0	1·2
	3·995	43·5	1·1
Tri-isobutylaluminium (neat)	2·050	91·0	4·5
	4·106	166·0	4·1
Di-n-propylaluminium isopropoxide (dilute solution in hydrocarbon) No. 1	3·580	85·0	2·3
	9·801	227·0	2·3
Di-n-propylaluminium isopropoxide (dilute solution in hydrocarbon) No. 2	0·427	105·6	24·3
	3·590	900·0	24·6

Table IV

Results for di-n-propylaluminium isopropoxide

Sample No.	1	2	3
Isopropoxide content found, %	39·7	32·0	31·1
n-Propyl content found, % ..	45·1	51·9	52·9
Aluminium content found, % ..	15·4	15·6	15·4
Sum of contents, %	100·2	99·5	99·4
Empirical formula	$Al_{1·00}Pr_{1·84}(OPr)_{1·18}$	$Al_{1·00}Pr_{2·08}(OPr)_{0·94}$	$Al_{1·00}Pr_{2·14}(OPr)_{0·92}$
Sum of organic subscripts in empirical formula	3·02	3·02	3·06
Theoretical sum of subscripts ..	3·00	3·00	3·00
Difference from theoretical, %	+0·6	+0·6	+2·0

the weight of alcohol isolated is proportional, within reasonable limits, to the weight of sample; similar results for alkoxide content are obtained, therefore, regardless of the amount of sample taken. As both the sources of error previously discussed would be expected to occur randomly, the good reproducibility obtained indicates that these errors are not involved to any appreciable extent in the determination.

A further check on the accuracy of the proposed method was made by determining the isopropoxide contents of three samples of di-n-propylaluminium isopropoxide. The results, together with the accurately determined aluminium and n-propyl-group contents, are shown in Table IV; the calculated empirical formulae for the samples are also shown. It can be seen that the sum of the subscripts in each empirical formula is always within 2 per cent. of the expected value, indicating that a satisfactory degree of accuracy was attained in determining the alkoxide groups.

I thank the Directors of Shell Chemical Company Limited for permission to publish this paper.

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The Determination of Gold in Mill Products by Means of a Cathode-ray Polarograph

By K. J. CATHRO

(*C.S.I.R.O., Chemical Research Laboratories, Box* 4331, *G.P.O., Melbourne, Australia*)

A method is described for determining gold in cyanide leach solutions, concentrates and calcines with use of a cathode-ray polarograph. The gold is precipitated from hydrochloric acid solution with stannous chloride solution, tellurium being used as a carrier. The tellurium - gold precipitate is redissolved, and the gold is determined in an ethylenediamine tartrate - potassium pyrophosphate base electrolyte. Large amounts of copper interfere with the polarographic determination, but the co-precipitation of gold on tellurium separates the gold from most of any copper present. The results agree well with those obtained by fire assay; a coefficient of variation of ± 3 per cent. is attained with 100 to 500 μg of gold.

During an investigation of the rate at which gold dissolved in cyanide solutions, it became necessary to determine gold in a large number of such solutions. No facilities for fire assay were available, and, as only small amounts (about 0·1 mg) of gold were to be determined, an instrumental method was sought. Tucker[1] has proposed a polarographic method for determining small amounts of gold in the "tailing" solutions from the precipitation section of a cyanidation plant. These solutions contain about 0·5 mg of gold per litre, and Tucker's method required a sample containing 17 to 85 μg of gold. No information was given about the precision or accuracy of the method, and the results found polarographically were not compared with those obtained by a more conventional method.

Several spectrophotometric methods have been suggested for determining gold; these involve use of reagents such as *p*-dimethylaminobenzylidenerhodanine,[2,3] bromide,[4,5] methyl violet,[6] *o*-tolidine,[7,8] dithizone[9] and rhodamine B.[10,11] Cyanide interferes with all these methods, and it is necessary to separate the gold from a cyanide solution before any of them can be applied. From the information available in the literature, the method based on

extraction of rhodamine B chloroaurate into benzene and measurement of the optical density of the extract seemed to be among the most suitable for our purpose, but preliminary tests showed that the coefficient of variation for this method over its best range was approximately ± 5 per cent. As a cathode-ray polarograph was available, it was decided to ascertain whether or not a more precise polarographic method could be developed.

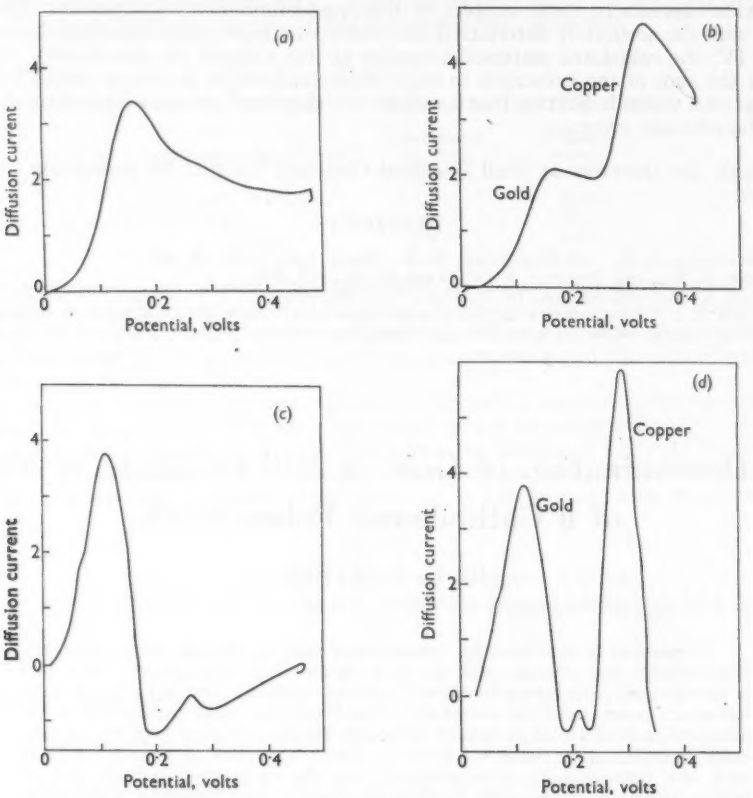

Fig. 1. Cathode-ray polarograms recorded at 30° C in the 0·5 M ethylenediamine tartrate - 0·1 M potassium pyrophosphate base electrolyte, with start potential of zero volts against a saturated-calomel electrode: (*a*) solution containing 19·8 μg of gold per ml (direct circuit; scale factor 0·6); (*b*) solution containing 19·8 μg of gold and 10 μg of copper per ml (direct circuit; scale factor 1·0); (*c*) solution containing 19·8 μg of gold per ml (derivative circuit; scale factor 0·006); (*d*) solution containing 19·8 μg of gold and 10 μg of copper per ml (derivative circuit; scale factor 0·006)

Experimental

Interference of copper in polarography of gold—

Preliminary investigations showed that gold gave an analytically useful wave in hydroxide medium, as reported by Hermann.[12] This wave was well formed with both conventional and cathode-ray polarographs, but, unfortunately, any copper present was reduced at the same potential as the gold, thereby interfering with the determination of the latter. As many mill solutions contain much greater amounts of copper than of gold, it was necessary to find a more suitable base electrolyte.

Tucker[1] determined gold polarographically in a base electrolyte 2·5 M in sodium hyroxide after extraction with ethyl acetate or ion exchange had been used to separate the gold from

interfering elements. Extraction with ethyl acetate as a means of separating copper from gold was tried in this laboratory, but it was found that recovery of gold at the 100-μg level was only about 90 per cent. when a technique similar to that described by Tucker was used; further, the solvent-extraction stage was slow when applied to a large batch of samples.

Beran and his co-workers[13] had suggested the use of base electrolytes containing ethylenediamine tartrate in the determination of gold; a well formed wave produced by reduction of auric gold was observed at approximately —0·08 volt against a saturated-calomel electrode. Chloride ion was reported to interfere seriously with the wave for gold, and no more than traces of chloride could be present. Číhalík, Doležal, Simon and Zýka[14] suggested that a base electrolyte containing 0·6 M ethylenediamine tartrate and 0·1 M potassium pyrophosphate was suitable for determining gold in presence of copper and iron; the incorporation of pyrophosphate caused the waves for copper and iron to shift to more negative potentials. Tests made with a base electrolyte of this type showed waves at —0·06, —0·20, and —0·43 volt against a saturated-calomel electrode for gold, copper and iron, respectively. In practice, it was found that the half-wave potential for gold depended on the purity of the ethylenediamine tartrate; when laboratory-reagent grade material was used, the observed half-wave potential was — 0·10 volt against a saturated-calomel electrode, whereas with material freshly prepared from anhydrous ethylenediamine and analytical-reagent grade tartaric acid, the half-wave potential was —0·06 volt. With a conventional d.c. polarograph, the determination of gold in presence of an equal weight of copper or up to five times as much iron was possible under optimum conditions. Consequently, a brief study of the reduction of gold and copper ions in ethylenediamine tartrate - potassium pyrophosphate base electrolyte was made with use of a cathode-ray polarograph.

This study showed that increases in the concentration of ethylenediamine tartrate over the range 0·1 to 1·0 M improved the definition of the peak for gold, whereas resolution of the peaks for gold and copper was better at the lower concentrations of ethylenediamine tartrate; the optimum concentration for the determination was about 0·5 M. Similarly, as the pH was increased, resolution of the peaks for gold and copper was improved, but the wave for gold became more poorly defined; the optimum pH of the base electrolyte was found to be about 5·8. When a base electrolyte 0·5 M in ethylenediamine tartrate, 0·1 M in potassium pyrophosphate and having a pH of 5·8 was used, a smooth-peaked wave was produced on the cathode-ray polarogram; the relationship between peak height and concentration of gold was linear over the range 10^{-5} to $2{\cdot}5 \times 10^{-4}$ M (2 to 50 μg of gold per ml) when the direct circuit was used. When the derivative circuit was used, the relationship between peak height and concentration of gold was no longer linear, and a calibration graph was required. With freshly prepared ethylenediamine tartrate, the peak potential was —0·17 volt against a saturated-calomel electrode (direct circuit) or —0·12 volt (derivative circuit), and it was possible to determine the height of the peak for gold by using the direct circuit, provided that the ratio of copper to gold did not exceed 0·5 to 1. With the derivative circuit, the peak for gold was clearly defined at ratios of copper to gold not exceeding 3 to 1 (see Fig. 1). The presence of iron^{III}, silver, arsenic^{III}, tellurium^{IV}, selenium^{IV} or antimony^{III}—the elements liable to be co-precipitated or reduced to metal by stannous chloride—in amounts ten times that of the gold produced no change in the peak height for gold; all these elements, except silver, are reduced at much more negative potentials than is gold. The silver is reduced at the start potential, so that a large ratio of silver to gold would interfere with the determination.

Recovery of gold from cyanide solutions—

Precipitation in presence of tellurium separates gold from most of the copper present in a sample,[15] and two series of tests were made to check the recovery of gold from cyanide solutions in presence of copper. In the first series, gold was recovered from prepared solutions containing 100 μg of gold, between 1 and 100 mg of copper and 0·25 per cent. of potassium cyanide and 0·01 M in hydroxide; the results were—

Copper present, mg	1	5	10	100
Gold found in precipitated tellurium, μg	104	102	98	105
Copper found in precipitated tellurium, μg	5	9	7	260

In the second series, known amounts of gold added to a mill solution (containing 122 mg of copper and 58 mg of iron per litre and amounts of zinc, cobalt, and nickel similar to that

of the gold initially present) were recovered; for 5-ml portions of the mill solution, the results were—

Gold added, μg	Nil	20	50	100	200
Gold found, μg	55	75	108	158	252
Recovery of added gold, μg	—	20	53	103	197

In both series of experiments gold was precipitated, together with tellurium as carrier, from hydrochloric acid solution by stannous chloride and determined polarographically in ethylenediamine tartrate - potassium pyrophosphate supporting electrolyte. The results clearly show that gold can be determined in the presence of a 1000-fold excess of copper if the gold is precipitated from acid solution by stannous chloride, with tellurium as carrier, and that none of the impurities present in a typical mill solution interferes with the determination.

Determination of gold in mill products—

With mill solutions, a dark precipitate often appeared when the solution was acidified before precipitation of gold by stannous chloride. This precipitate contained copper and was carried down with the gold - tellurium precipitate, thereby heavily contaminating the latter with copper. The precipitate obtained when the solution was acidified was attributed to the presence of anions, such as thiosulphate or xanthate, in the mill solution; to prevent formation of such a precipitate, the solution was treated with alkaline hypobromite before acidification in order to oxidise any of the sulphur compounds to sulphate. When this was done, the solution obtained on acidification was clear and the amount of copper co-precipitated with the gold and tellurium was greatly decreased.

Method

Apparatus—

Most of the polarographic studies were made with use of a K1000 cathode-ray polarograph (Southern Analytical Ltd.). A limited amount of work was carried out with a Leeds - Northrup Electrochemograph, type E (a conventional d.c. polarograph). Pot-type cells, with provision for de-gassing, were used with the K1000 polarograph in conjunction with an amalgamated-silver-wire anode; an H-cell incorporating a saturated-calomel electrode was used with the Electrochemograph. Spectrophotometric determinations were carried out with use of a Uvispek spectrophotometer and 10-mm glass cuvettes.

Reagents—

When available, materials of recognised analytical grade were used without further purification.

Potassium pyrophosphate—Heat 250 g of the best available grade of dipotassium hydrogen orthophosphate at 600° C for 5 hours, allow the calcined product to cool in a desiccator, and store it in a tightly sealed bottle.

Ethylenediamine tartrate - potassium pyrophosphate base electrolyte—To 50 ml of chilled 2 M tartaric acid add 6·4 ml of anhydrous ethylenediamine, with constant stirring. Dissolve approximately 6·6 g of the potassium pyrophosphate in this solution, dilute to 90 ml, and cool to room temperature. Adjust the pH to 5·8 (check with a glass-electrode pH meter) with dilute sulphuric acid or ammonia, and dilute to 100 ml. This solution is approximately 1 M in ethylenediamine tartrate and 0·2 M in potassium pyrophosphate; prepare a fresh batch daily, as required.

Standard gold solution—Dissolve 1 g of chloroauric acid in 500 ml of distilled water, and store in a tightly stoppered dark-glass bottle. Prepare more dilute standard solutions, as required, from this concentrated solution. Check the concentration of gold in this solution gravimetrically.[16]

Tellurium tetrachloride solution—Dissolve 1 g of elemental tellurium in aqua regia, evaporate to dryness at as low a temperature as possible, and dissolve the residue in 1 litre of 2 M hydrochloric acid.

Hydrochloric acid, 6 M—Prepare by diluting the concentrated acid; check the molarity volumetrically, and adjust it, if necessary, to 6 ± 0·05.

Rhodamine B solution—Dissolve 0·2 g of the best available grade of rhodamine B in 100 ml of water.

RECOVERY OF GOLD FROM CYANIDE SOLUTION—

To a suitable aliquot of the cyanide solution (containing not less than 20 and preferably more than 100 μg of gold) add sufficient hydrochloric acid to make the final solution 2 M in this acid. If the addition of acid causes formation of a dark precipitate add 2·5 ml of 0·5 M sodium hypobromite for each 10 ml of sample solution present before the addition of acid. (At this stage, the solution should be clear, or, if hypobromite has been added, yellow to orange from liberated bromine.) Add 1 ml of tellurium tetrachloride solution, heat to approximately 80° C, and add sufficient of a 20 per cent. solution of stannous chloride in 2 M hydrochloric acid to precipitate the tellurium completely (1 to 5 ml will usually be necessary). Dilute to 60 ml, allow the black precipitate to coagulate (this takes about 15 minutes), and collect it on a Whatman No. 541 filter-paper. Wash the precipitate twice with 1 M hydrochloric acid and once with hot water, transfer filter-paper and precipitate to a borosilicate-glass 10-ml beaker, dry at 110° to 160° C, and ignite at 500° to 550° C to remove all organic matter. Continue as described under "Polarographic Procedure."

TREATMENT OF SOLID MILL PRODUCTS—

The analysis of solid mill products requires solution of the sample by a suitable technique. In general, bring from 1 to 5 g of sample into solution by heating with 20 ml of aqua regia. Add 5 ml of sulphuric acid, and evaporate until dense white fumes are evolved. Cool, dissolve soluble salts in the minimum volume of brominated 2 M hydrochloric acid, filter the solution through a Whatman No. 540 filter-paper, and wash the residue with 1 M hydrochloric acid. If the residue is suspected to contain gold, ignite it to burn off the filter-paper, and then fuse with sodium carbonate. Acidify the melt with hydrochloric acid, evaporate to dryness, dissolve soluble salts in brominated hydrochloric acid, and filter as before. Combine both filtrates for the determination of gold. (Note that this technique may be inconvenient for application to highly siliceous samples; for such samples, an initial attack with hydrofluoric acid, as suggested by Onishi,[11] might be better.)

When the sample has been brought into solution, add tellurium tetrachloride solution, and use stannous chloride to precipitate the gold on tellurium as described above (the precipitate coagulates slowly in presence of much iron or copper). Separate, wash, dry and ignite the precipitate as before, and continue as described under "Polarographic Procedure."

POLAROGRAPHIC PROCEDURE—

Dissolve the calcined material in 0·5 ml of a mixture of equal volumes of concentrated nitric and hydrochloric acids, add about 0·5 ml of water, and evaporate to dryness under an infra-red reflector lamp. (The evaporation must leave a dry residue containing no more than traces of chloride, but this residue must not be baked, or some gold might be reduced to metal.) Add 5 ml of the ethylenediamine tartrate - sodium pyrophosphate base electrolyte to the dry residue, and set aside for about 5 minutes. Transfer the solution to a 10-ml calibrated flask, rinse the beaker twice with water, and add the rinsings to the contents of the calibrated flask. Dilute to the mark, insert the stopper, and mix; if the solution is very cloudy (it is rarely perfectly clear), clarify by centrifugation. Finally, transfer the solution to a polarographic cell, de-aerate for not less than 5 minutes by passage of nitrogen, and record a polarogram; use a start potential of −0·10 volt against the silver-wire anode (zero volts against a saturated-calomel electrode). Plot a calibration graph from the results obtained for standards covering the range of expected concentrations of gold. If much copper is present, use the derivative circuit of the cathode-ray polarograph to resolve the peak for gold. If a conventional d.c. polarograph is used, the polarogram should be recorded between +0·1 and −0·3 volt against a saturated-calomel electrode; the half-wave potential is −0·06 volt against a saturated-calomel electrode.

PHOTOMETRIC DETERMINATION—

Take a sample containing from 5 to 30 μg of gold, and concentrate the gold by co-precipitation with elemental tellurium as described above. Dissolve the ignited precipitate in 1 or 2 drops of a mixture of equal volumes of concentrated nitric and hydrochloric acids, heat to ensure solution of the gold, and transfer to a 25-ml calibrated flask. By pipette, add 10 ml of 6 M hydrochloric acid and then 1 ml of 0·2 per cent. rhodamine B solution, dilute to 20 ml, insert a stopper, and mix. Immediately extract the rhodamine B chloroaurate by shaking with 10·0 ml of benzene for 45 to 60 seconds. Allow the organic layer to

separate, remove it from the separating funnel, and spin in a centrifuge for a minute or two to ensure complete separation from the aqueous phase. Measure the optical density of the extract at 565 mμ against a reagent blank solution, and determine the amount of gold present by reference to a calibration graph prepared from the results obtained for known amounts of gold in the range 5 to 30 μg.

Results

Comparison between polarographic, spectrophotometric and fire-assay methods—

The proposed method was tested by comparing the results obtained polarographically with those obtained by fire assay and by a spectrophotometric method involving use of rhodamine B and developed from that described by MacNulty and Woollard.[10] The samples used were (*a*) mill solutions containing considerable amounts of copper (0·05 to 2 g per litre) and concentrations of zinc, cobalt, nickel and silver similar to that of the gold, (*b*) solid mill products comprising pyritic concentrates containing about 30 to 35 per cent. of iron, 35 per

Table I

Gold found in mill cyanide solutions

The numbers of determinations made by the polarographic and spectrophotometric methods are indicated by the figures in parenthesis

Sample*	Copper content, mg per litre	Silver content, mg per litre	Gold content found by— fire assay, mg per litre	polarography, mg per litre	spectrophotometry, mg per litre
GB Precyanide solution ..	455	4	7·4	7·6 (2)	7·4 (3)
GB Calcine solution ..	1955	14	90	91 (2)	91 (4)
LVS Calcine solution A ..	356	4	62	66 (6)	67 (4)
LVS Postcyanide solution ..	50	0·4	0·4	0·6 (2)	0·6 (1)
LVS Calcine solution B ..	122	0·6	10·7	10·6 (10)	10·5 (2)
GMK Calcine solution ..	784	2	16·5	16·9 (2)	16·7 (4)
GMK Concentrate solution ..	460	39	155	157 (2)	156 (4)
NK Concentrate solution ..	360	20	65	66 (2)	63 (4)
NK Calcine solution	648	12	31	31 (2)	31 (4)

* GB = Great Boulder Mines Ltd.
LVS = Lake View and Star Ltd.
GMK = Gold Mines of Kalgoorlie (Australia) Ltd.
NK = North Kalgurli (1912) Ltd.

Table II

Gold found in solid mill products

The numbers of determinations made by the polarographic and spectrophotometric methods are indicated by the figures in parenthesis

Sample*	Copper content, p.p.m.	Silver content, p.p.m.	Gold found by— fire assay, p.p.m.	polarography, p.p.m.	spectrophotometry, p.p.m.
NK Roaster feed	950	23	23	25 (2)	27 (4)
NK Roaster discharge	1325	29	38	36 (4)	46 (4)
NK Cleaner flotation concentrate ..	1270	51	88	84 (4)	—
NK Calcine cyanidation residue ..	1025	22	3·2	4 (2)	8 (4)
GB Flotation concentrate	3100	119	121	118 (2)	126 (4)
LVS Calcine	1000	37	117	115 (2)	127 (4)
LVS Concentrate	830	33	110	106 (3)	120 (4)

* See foot-note to Table I.

cent. of sulphur, 25 per cent. of gangue minerals, 0·1 to 0·3 per cent. of copper and minor amounts of many other elements, (*c*) the calcines obtained by roasting these concentrates and (*d*) residues from cyanidation. The solid samples were brought into solution by treatment with aqua regia, fusion of any insoluble residue with sodium carbonate and extraction of the

fused product with acid; the various acid fractions were combined and used for the determination of gold. The results obtained are shown in Tables I and II. In general, these results are in good agreement with those obtained by fire assay, except for the values obtained spectrophotometrically for the solid products, which tended to be high. (This was probably because appreciable amounts of iron—about 0·1 to 1·0 mg—were carried down from these solutions by the tellurium - gold precipitate; iron would interfere with the rhodamine B determination, giving high results of the order observed.)

PRECISION—

The precision of the method was tested by subjecting ten aliquots of a prepared cyanide solution, each containing 110 μg of gold, to the entire procedure. The gold was recovered quantitatively, and the coefficient of variation for the determination was 3·4 per cent. when the direct circuit of the cathode-ray polarograph was used and 1·4 per cent. for the derivative circuit. A second series of ten tests was made on a plant solution, and only the direct circuit was used; the coefficient of variation was 3·2 per cent., a result in good agreement with those for the prepared solutions. As a final assessment of precision, the coefficient of variation was calculated from the agreement between the results of routine duplicate determinations, as suggested by Stace and Hutton.[17] The results are shown in Table III, from which it can be seen that the coefficient of variation attained when a cathode-ray polarograph is used is

TABLE III

PRECISION OF ROUTINE DETERMINATIONS WITH CATHODE-RAY POLAROGRAPH

Amount of gold determined, μg	Direct circuit		Derivative circuit	
	Number of sets of duplicate determinations	Coefficient of variation, %	Number of sets of duplicate determinations	Coefficient of variation, %
100 to 200	41	2·8	30	2·7
200 to 300	27	2·5	17	2·4
300 to 600	11	1·8	—	—

about ±2·5 per cent. for amounts of gold greater than 100 μg. For amounts of gold less than this, the number of determinations made has been insufficient to ascertain the precision, but such tests as have been carried out indicate a precision of the order of ±5 per cent. in the range 20 to 100 μg of gold. These results may be compared to a coefficient of variation of 4·5 per cent. obtained by applying the rhodamine B method to a similar range of mill solutions. A conventional d.c. polarograph can be used in place of the cathode-ray instrument, but there is a loss of precision for samples containing less than 300 μg of gold; for 100 μg of gold, the coefficient of variation obtained when a d.c. polarograph was used was of the order of ±5 per cent.

CONCLUSIONS

Gold can be determined in a range of solid and liquid mill products by polarographic or spectrophotometric methods. In its best range—for amounts of gold greater than 100 μg —the cathode-ray polarographic procedure gives more precise results (coefficient of variation, about 2·5 per cent.) than does the photometric method (coefficient of variation, 4·5 per cent.) in its best range—10 to 30 μg of gold. Relatively large amounts of copper and silver interfere with the polarographic method; the photometric method is less susceptible to interference from these elements, but large amounts of iron cause high results. In the absence of interferences both methods give results in good agreement with those found by fire assay. There is little to choose between the two methods in speed, as both need about 25 minutes per determination when solutions are analysed in batches of twelve to twenty, although the time needed for a determination is markedly longer for solid samples, owing to the necessity for bringing them into solution.

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The Preparation of Small Rock Salt Plates for Micro-sampling in Infra-red Spectrophotometry

By S. F. Nicholls

(*The Cotton, Silk and Man-made Fibres Research Association, Shirley Institute, Didsbury, Manchester* 20)

A simple method is described for preparing rock salt plates a few square millimetres in area. The technique of using these plates as supports to obtain the infra-red spectra of very small samples is outlined and is illustrated by the spectra of dimethylformamide, beeswax and polyethylene fibre.

The problem of obtaining the spectrum of a small sample of a liquid or coherent solid when micro-sampling accessories are not available can often be solved with the help of rock salt plates a few square millimetres in area. These plates can be prepared in the laboratory from scrap window material, which is ground and polished and then cut to convenient size. The exacting procedure for grinding and polishing infra-red transparent material described by Strouts, Gilfillan and Wilson[1] is unnecessary when a high degree of optical flatness is not the primary consideration, and micro-plates satisfactory for semi-quantitative work can be prepared by a much simplified method.

Preparation of micro-plates

The scrap material, *e.g.*, discarded cell windows, is cleaved into fragments about 5 mm × 10 mm × 1·5 mm in size by tapping sharply on the back of a single-edged razor blade held with its cutting edge touching the material parallel to a cleavage plane. The faces are trimmed and ground approximately parallel on a roughened glass plate with fine carborundum (*e.g.*, grade No. 3F) and absolute ethanol by rubbing the rock salt with a circular motion under the finger-tip, which is protected by a rubber finger-cot. The micro-plates are washed with absolute ethanol and mounted in the well (about 0·1 inch deep) of a circular hardwood holder for grinding and polishing; a wax having a fairly low melting-point, *e.g.*, Sira wax, is suitable for mounting. To mount the micro-plates, a rectangular wooden

frame having a circular hole into which the holder can slide is laid on a sheet of plate glass, and an annulus of thin metal foil, with its inner diameter slightly smaller than that of the well of the holder, is put inside the hole. The rock salt plates are arranged on the circle of glass, and the holder, with its well partly full of almost molten wax is inverted over them (see Fig. 1). A weight is put on top, and the wax is allowed to set. Plates having different thicknesses can be accommodated at one time. The surfaces of the plates stand proud of the holder by the thickness of the foil and are ground down to the level of the rim on a slightly roughened piece of plate glass with absolute ethanol and fine carborundum. A suitable carborundum can be prepared by shaking commercial carborundum (grade 3F) with water in a tall cylinder and collecting the material still suspended in the water after 30 minutes. The plates are washed with absolute ethanol, and the grinding is repeated with fine rouge (*e.g.*, Cerirouge E) as abrasive. In both operations the work should be kept wet with lubricant.

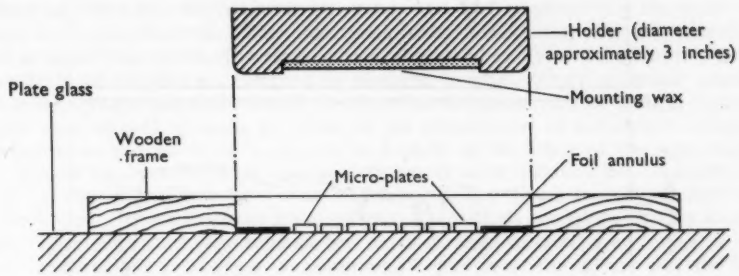

Fig. 1. Diagram showing technique for mounting micro-plates for grinding and polishing

Before being polished, the plates are washed completely free from abrasive with absolute ethanol. Polishing is best done on a pitch lap prepared as described by Strouts, Gilfillan and Wilson[1]; 200 g of the recommended pitch - resin mixture will make a lap 6 inches in diameter and 0·5 inch thick, which is supported on a wooden disc. Although the lap is somewhat troublesome to make, it imparts an excellent finish to the surfaces of the plates. The surface of the lap is moistened with a suspension of fine rouge in a saturated aqueous solution of sodium chloride, and the micro-plates are polished on the lap with a light circular motion repeated fifteen to twenty times and finished off on a dry velvet cloth stretched over a glass plate. The plates are lapped again if necessary and then detached from the holder by levering them out of the softened wax. The procedure is repeated for the opposite faces, and the edges of the plates are trimmed free from debris. Traces of the mounting wax are removed by a final buffing, which is carried out by gripping the micro-plates by their edges with plastic forceps and rubbing them with a circular motion on the polishing cloth. Good contact between the polishing cloth and the surface of the micro-plate is obtained by putting a piece of cork on top of the micro-plate and pressing on it with the finger. The micro-plates can then be cleaved into smaller pieces; 5 mm × 2 mm and 10 mm × 2 mm are useful sizes.

Measurement of spectra

When the sample is solid, it is deposited from solution as a film on the warm micro-plate resting on a small levelled hot stage. The last traces of solvents having high boiling-points are removed by heating the prepared film *in vacuo*. To obtain the spectrum, the micro-plate is temporarily mounted with plasticine over a slot cut in a metal plate that slides into a conventional cell holder. When the sample is a liquid, it is best to mount one micro-plate first, deposit the film on it, and then put a second micro-plate on top.

If the micro-plates obscure the sample slit, some form of reference-beam attenuator will be found useful to compensate for energy losses. Several auxiliary methods of attenuating the beam have been described, including the rotating starwheel[2] and triangular comb,[3] both of which can give infinitely variable attentuation. In this laboratory, wire gauzes having open areas ranging from 65 to 25 per cent. are used. Each is mounted over a hole in a thin card, which slides into a conventional cell holder; the range of upper-scale expansion that they provide (× 1·5 to × 4) has been found sufficiently great for most purposes.

Fig. 2 shows spectra that indicate some applications of these micro-plates. The sample of beeswax was applied as a film from methylene chloride solution; the film was then melted and allowed to cool slowly. The Courlene (polyethylene) fibre was dissolved in boiling xylene and applied as a film; the plate was then heated *in vacuo* at about 120° C for 30 minutes. The spectra were recorded with an Infracord spectrophotometer equipped with a rock salt prism.

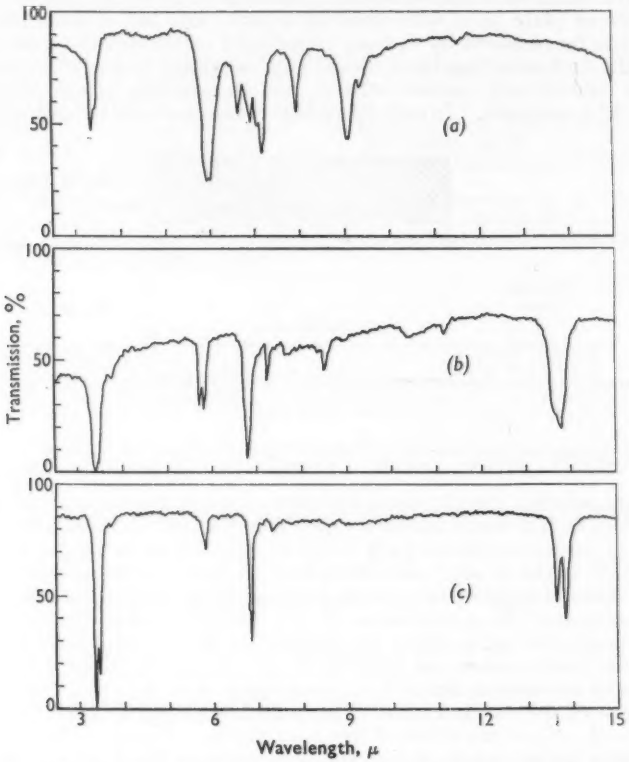

Fig. 2. Spectra of samples deposited as films on micro-plates: (*a*) about 0·2 μl of dimethylformamide on plate 2 mm × 10 mm (no screen); (*b*) about 0·5 mg of beeswax on plate 2 mm × 5 mm (0·4 open-area screen); (*c*) about 2 mg of Courlene (polyethylene) fibre on plate 5 mm × 10 mm (no screen)

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Notes

CHANGES IN THE COMPOSITION OF THE CREAM FILLING OF SPONGES ON STORAGE

In my capacity as Public Analyst, I had submitted to me by a Local Authority an informal sample consisting of a sponge sandwich filled with, according to the declaration, "Fresh Dairy Cream." Each sandwich consisted of two discs of sponge mixture with a layer of cream between; there was also a thin layer of jam between each disc of sponge and the cream. Each sponge disc weighed about 70 g, each layer of jam about 10 g and the cream filling about 100 g. I found that the cream filling contained about 10 per cent. of sucrose, and I raised the point that the presence of the sugar in the filling should have been declared. The Local Authority referred the matter to the manufacturers, who replied that they did not put any sugar into the cream filling. Further investigation showed that the sugar was derived from the sponge and had migrated into the cream filling after the sponges were manufactured. They were quick-frozen immediately after manufacture and were contained in sealed bags made of moisture-proof cellulose film. The manufacturers kindly agreed to co-operate in an experiment designed to discover to what extent the sugar passed into the filling while the sponges were cold-stored, how much subsequent transfer of sugar took place when the sponges were allowed to warm up either to the temperature of a domestic refrigerator or to room temperature, and what other changes took place in the filling at the same time.

The sponges are manufactured and quick-frozen at some distance from Norwich, and it was inevitable that there should be a delay of about 2 days between their leaving the factory and arriving in this laboratory. It was therefore arranged that the sponges should be sent by passenger train in insulated containers filled with solid carbon dioxide; when received they were still frozen solid. They were accompanied by a glass jar containing a sample of the cream used for filling them, also frozen. This sample of cream, and the fillings from two of the sponges (excluding the jam), were analysed immediately on receipt. Some of the sponges were held for 24 and 48 hours at room temperature (about 65° F) and others were kept in a domestic refrigerator at about 40° F for 24, 48, 72 and 96 hours, and were then analysed, the fillings from two sponges being combined for each analysis.

Some sponges from the same batch, together with another jar of cream, were held at the factory in cold-store for a week, and were then sent to Norwich in the same way as before. The analytical procedure with the second batch was identical with that of the first.

TABLE I

ANALYSIS OF CREAM FILLING FROM SPONGE SANDWICHES, HELD DEEP-FROZEN FOR 2 DAYS

	On receipt		After storage at room temperature for—		After storage in a refrigerator for—			
	Before filling, %	After filling, %	24 hours, %	48 hours, %	24 hours, %	48 hours, %	72 hours, %	96 hours, %
Water ..	56·8	56·9	44·1	37·7	51·7	48·9	46·3	42·0
Fat ..	38·1	35·0	42·5	44·4	36·5	37·8	36·7	39·1
Sucrose ..	Nil	0·5	4·9	9·2	5·4	5·8	7·9	10·1
Invert sugar	Nil	1·4	1·3	1·8	1·5	1·3	1·7	2·1
M.S.N.F. ..	5·3	6·2	7·2	6·9	4·9	6·2	7·4	6·7

TABLE II

ANALYSIS OF CREAM FILLING FROM SPONGE SANDWICHES, HELD DEEP-FROZEN FOR 9 DAYS

	On receipt		After storage at room temperature for—		After storage in a refrigerator for—			
	Before filling, %	After filling, %	24 hours, %	48 hours, %	24 hours, %	48 hours, %	72 hours, %	96 hours, %
Water ..	57·1	57·7	47·1	41·1	51·0	48·4	45·9	42·0
Fat ..	38·1	36·1	42·1	44·3	37·4	37·3	38·4	39·5
Sucrose ..	Nil	0·8	3·8	6·3	3·2	5·7	6·6	7·6
Invert sugar	Nil	0·7	1·1	1·2	0·9	1·4	1·3	1·5
M.S.N.F. ..	5·2	4·7	5·9	7·1	7·5	7·2	7·8	9·4

The results of all these analyses are shown in Tables I and II. Water and fat were determined by conventional methods. Sucrose, invert sugar and lactose were determined by observing the polarisation before and after inversion and the reducing power with Fehling's solution. It was then assumed that no substances having either optical rotation or reducing power were present other than the three substances named, and the corresponding equations were solved. On the samples of cream in the glass jars, protein and ash were also determined, and the quantity referred to as Milk Solids Not Fat (M.S.N.F.) in the Tables is, for these samples, the sum of the lactose, ash and protein. This is the reason why these two analyses do not add up to exactly 100 per cent. With all other samples, the figure recorded as M.S.N.F. was determined by difference to reduce the amount of analytical work.

From the results shown in Tables I and II, the following conclusions can be drawn—

1. Even while the sponges are deep-frozen, a little sugar passes into the cream filling, either from the jam or from the sponge or both. The fact that the amount of sucrose *plus* invert sugar was found to be 1·9 per cent. after 2 days and 1·5 per cent. after 9 days is probably more a reflection of the analytical error of these analyses than anything else. Since the sugars are determined by solving three simultaneous equations, small errors in the observed polarimetric readings or the reducing power give rise to relatively large errors in the percentage of sucrose, invert sugar or lactose. Similar errors will occur in the M.S.N.F., since this figure is obtained by difference.

2. When the sponges are allowed to thaw, migration of sucrose or invert sugar into the cream increases considerably. The extent of this migration can be best seen from the figures of Table III, in which the analyses have been based on dry matter to remove the effect of the variations in water content (see below). After 48 hours at room temperature, as much as 17 per cent. of sucrose *plus* invert sugar may be present in the dry solids of the cream, and after 96 hours in a domestic refrigerator, as much as 21 per cent. I cannot say why the percentage of sugar migrating under any given set of conditions should have been uniformly greater in the first batch of sponges than in the second, unless it is that after 9 days in a cold store, the sponge itself is altered so that the sugar it contains is more closely bound and less able to pass into the cream.

3. The moisture content of the cream filling remains constant during the deep-frozen period, but rapidly decreases as soon as the sponges are allowed to thaw, either by evaporation or (more probably) by absorption into the sponge.

TABLE III

ANALYSIS OF DRY MATTER OF CREAM FILLING

	On receipt		After storage at room temperature for—		After storage in a refrigerator for—			
	Before filling, %	After filling, %	24 hours, %	48 hours, %	24 hours, %	48 hours, %	72 hours, %	96 hours, %
Deep-frozen for 2 days—								
Fat ..	88·2	81·2	76·0	71·2	75·6	73·9	68·4	67·4
Sucrose and invert sugar	Nil	4·4	11·1	17·7	14·3	13·9	17·9	21·0
M.S.N.F. ..	12·3	14·4	12·9	11·1	10·1	12·1	13·8	11·6
Deep-frozen for 9 days—								
Fat ..	88·8	85·4	79·6	75·3	76·3	72·3	71·0	68·1
Sucrose and invert sugar	Nil	3·5	9·3	12·7	8·4	13·8	14·6	15·7
M.S.N.F. ..	12·1	11·1	11·2	12·1	15·3	14·0	14·4	16·2

These analytical findings raise interesting legal problems. The presence of sugar without declaration in a product sold as "cream" is not an offence under the Labelling of Food Order in this particular case, because flour confectionery is exempt from the labelling requirements of that Order. It might be regarded as an offence, however, against Section 47 of the Food and Drugs Act, since a mixture of cream and sugar is not cream and is being sold as such. Admittedly, it might be pedantic to object to the presence in the cream of a mere 1 or 2 per cent. of sugar, which is all that is present at the time of sale; but the principle must not be carried too far, since it would clearly be illegitimate to sell as cream a mixture of, say, 75 per cent. of cream and 25 per cent. of sugar. If the case were taken under Section 2 of the Food and Drugs Act, the charge being that

the article was not of the nature, substance or quality demanded by the purchaser, there would be an adequate defence under Section 3 (3), because the presence of the "extraneous matter," namely the sugar, is certainly in the present instance "an unavoidable consequence of the process of . . . preparation." This defence is not available under Section 47.

The further point arises, however, that unless special precautions are taken by the Sampling Officer, the frozen sponge will have thawed out by the time the Public Analyst receives it, and even if the analysis is begun at once, an appreciable amount of sugar may have migrated into the cream. The analyst under these circumstances may well assume, as I did before this investigation was made, that the sugar had been added to the cream by the manufacturer before the sponge sandwich was filled. In my opinion, the presence of as much as 12 per cent. of sucrose *plus* invert sugar in the cream, as found in one of the samples in Table I, would be an offence if it had been deliberately added without declaration; but surely there can be no offence if this amount of sugar passes into the cream as a natural consequence of the lapse of time between manufacture and consumption.

I thank Mr. M. R. Barker for carrying out the analyses referred to in this Note, and the kind co-operation of Birds Eye Foods Ltd. is gratefully acknowledged.

CLARENCE HOUSE
6, CLARENCE ROAD
NORWICH

ERIC C. WOOD
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THE MICROBIOLOGICAL ASSAY OF METHICILLIN AND PHENETHICILLIN BY LARGE-PLATE METHODS

METHICILLIN, *i.e.*, 6-(2,6-dimethoxybenzamido)penicillanic acid, and its salts and phenethicillin (α-phenoxyethylpenicillin) and its salts, both new penicillins prepared by chemical synthesis from 6-aminopenicillanic acid,[1] have been regularly assayed in these laboratories for more than a year. Large-plate methods have been used with strains of *Bacillus subtilis*, *Staphylococcus aureus* and *Sarcina lutea*.

METHICILLIN—

It has been suggested that the activity of new penicillins should be expressed in units of benzylpenicillin,[2] and this might be possible were the dose-response curves linear and parallel. If such units were to be used, the assay organism would need to be specified with each unit quoted, as the activity of a new penicillin would not necessarily be in the same ratio to that of benzylpenicillin for all assay organisms.

Methicillin has been assayed against sodium benzylpenicillin, and the regression equations, in which d is the mean diameter of the zone in millimetres and c is the concentration of antibiotic in micrograms per millilitre, are shown below. Equations (1), (2) and (3) are for the assay of benzylpenicillin with *B. subtilis*, *Staph. aureus* and *S. lutea*, respectively, as assay organism.

$$d = 24{\cdot}070 + 8{\cdot}438 \log c \quad .. \quad .. \quad .. \quad .. \quad (1)$$
$$d = 25{\cdot}780 + 13{\cdot}354 \log c \quad .. \quad .. \quad .. \quad .. \quad (2)$$
$$d = 30{\cdot}160 + 14{\cdot}085 \log c \quad .. \quad .. \quad .. \quad .. \quad (3)$$

Equations (4), (5) and (6) are for the assay of methicillin with *B. subtilis*, *Staph. aureus* and *S. lutea*, respectively, as assay organism.

$$d = 16{\cdot}362 + 13{\cdot}228 \log c \quad .. \quad .. \quad .. \quad .. \quad (4)$$
$$d = 2{\cdot}585 + 17{\cdot}579 \log c \quad .. \quad .. \quad .. \quad .. \quad (5)$$
$$d = 21{\cdot}000 + 16{\cdot}610 \log c \quad .. \quad .. \quad .. \quad .. \quad (6)$$

The relatively high assay levels for methicillin with *Staph. aureus* can be seen from the intercept of d on c in equation (5).

In no instance was the dose-response curve for methicillin parallel to that for benzylpenicillin, so that the impossibility of expressing methicillin in terms of units of benzylpenicillin is clear.

PHENETHICILLIN—

Phenethicillin consists of the two diastereoisomers, D-α-phenoxyethylpenicillin (D-isomer) and L-α-phenoxyethylpenicillin (L-isomer), with not less than 55 per cent. of the L-isomer. The

two isomers and phenethicillin and benzylpenicillin have been assayed by the plate methods described below. The dose-response curves for the D- and L-isomers and phenethicillin and benzylpenicillin were parallel for all three assay organisms, but the concentrations of the two isomers necessary to produce the same zones of inhibition were widely different. The ratio of the activities of the D- and L-isomers varied with the test organism, but a higher concentration of the D-isomer was always required. The D-isomer showed 17·0 per cent. of the activity of the L-isomer for *B. subtilis* (standard deviation for nine results, 0·94), 33·9 per cent. for *Staph. aureus* (standard deviation for seven results, 1·67) and 14·3 per cent. for *S. lutea* (standard deviation for ten results, 0·79). Further work is in progress on the activities of the two isomers in diffusion assays.

TEST ORGANISMS AND METHODS—

The assay media used were: for *B. subtilis*, either Oxoid nutrient agar No. 2 or the medium described by Price and Boucher[3]; for *Staph. aureus*, either Oxoid nutrient agar No. 2 or a buffered nutrient agar,[4] and for *S. lutea* the medium of Simpson and Lees,[5] with Oxoid agar. Single-layer plates were used for all assays, with an 8 × 8 randomised Latin-square design and either beads[6] on the surface or 10-mm holes for a cup-plate assay; solutions were added in horizontal row order.[2,7] Methicillin and phenethicillin were diluted for assay in 0·05 M phosphate buffer (pH 7·0).

ASSAY RANGES FOR METHICILLIN AND PHENETHICILLIN—

B. subtilis—The assay ranges were similar for both strains and media, but strain ATCC 6633 was preferred because it was easier to prepare as a spore suspension than was strain NCTC 8236, which tended to "clump" badly.

Staph. aureus—There was little difference between the assay ranges for either medium with the Oxford strain (NCTC 6571) or strain 209P (NCIB 8588); the Oxford strain was mostly used.

S. lutea—Suspensions prepared from slopes, not more than 2 weeks old, gave similar assay results with two strains, NCIB 8553 and ATCC 9341.

The assay levels and ranges are shown in Table I, which also includes the limits of error obtained in routine assays when two weighings of a standard preparation and two of a sample were included on one 8 × 8 plate. The standard used in methicillin assay had an assigned potency, by chemical assay, of 97·4 per cent. as the sodium salt monohydrate. The phenethicillin standard had an assigned potency, by chemical assay, of 99·7 per cent. as the potassium salt. The test samples were from the same batch of methicillin or phenethicillin for each of the three assay organisms.

TABLE I

ASSAY LEVELS AND LIMITS OF ERROR FOR METHICILLIN AND PHENETHICILLIN

Antibiotic	Assay organism	Rectilinear dose-response range, μg per ml	Assay levels μg per ml	Potency of test sample, %	Limits of error (P = 0·95)
Methicillin	*B. subtilis*	0·5 to 20	1·25 and 5·0	96·6	94·2 to 99·0
	Staph. aureus	3 to 30	5·0 and 20·0	96·5	94·2 to 98·8
	S. lutea	0·5 to 8	0·5 and 2·0	96·3	93·6 to 99·2
Phenethicillin	*B. subtilis*	0·5 to 20	1·25 and 5·0	98·9	95·5 to 102·4
	Staph. aureus	0·1 to 2	0·375 and 1·5	98·9	96·3 to 101·1
	S. lutea	0·05 to 1	0·05 and 0·2	99·0	95·5 to 102·6

We thank the Directors of Beecham Research Laboratories Ltd. for permission to publish.

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BEECHAM RESEARCH LABORATORIES LTD.
PRODUCT RESEARCH DIVISION
GREAT WEST ROAD
BRENTFORD, MIDDLESEX

A. JONES
R. M. PAY

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SEPARATION OF MIXTURES OF DRUGS BY A COMBINATION OF CHROMATOGRAPHY AND IONOPHORESIS ON CELLULOSIC ION-EXCHANGE SHEETS

THE recent production of modified cellulosic ion-exchange paper in sheet form by the manufacturers of Whatman filter-paper has opened up a new field of chromatography; so far, these papers seem to have been used only for the separation of metals and amino acids.[1] Because chromatography on such paper can be carried out with aqueous solutions and because the rate of separation is much greater than with ordinary filter-paper, it was considered that the papers would be of use in separating various mixtures of drugs. Further, it was thought that, by subjecting the papers to ionophoresis, the additional parameter of ion-exchange might effect separations in a different order from those with unmodified cellulose paper. In preliminary communications,[2] we have shown that mixtures of various drugs can be separated by this technique and that the time required for resolution is shorter than when conventional techniques are used. This Note describes the details of the technique we have used to effect separation of mixtures of (*a*) acetophenetidin, *p*-acetylaminophenol, phenobarbitone and salicylic acid and (*b*) acetophenetidin, sulphacetamide, quinine and promazine.

To avoid confusion, it must be emphasised that this work deals only with modified cellulose papers and not with filter-paper impregnated with ion-exchange resins or with ion-exchange membranes.

MATERIALS, APPARATUS AND REAGENTS

Paper—Whatman diethylaminoethylcellulose sheet (DE20) was used; it was cut into rectangles measuring 4 inches × 5 inches. Chromatography was carried out in the shorter direction and ionophoresis in the longer. Both processes were carried out in the same tank, *viz.*, a Shandon micro-electrophoresis tank, and the same solution was used for chromatography and ionophoresis.

Drugs—The drugs were used as 2 per cent. w/v solutions in water, ethanol or chloroform; spots containing about 2 to 5 μl of drug solution were placed on the paper.

Ammonia solution, 0·2 N.

EDTA solution, 0·1 M—Prepared from the disodium salt of ethylenediaminetetra-acetic acid.

SEPARATION OF MIXTURE OF ACETOPHENETIDIN, *p*-ACETYLAMINOPHENOL, PHENOBARBITONE AND SALICYLIC ACID

In toxicological analysis, the acidic and neutral fractions may contain acetophenetidin. According to Stewart and Stolman,[3] the major metabolite of acetophenetidin in man is *p*-acetylaminophenol, which might therefore be present in an ether extract of an acidic blood filtrate.

About 2 μl each of 2 per cent. solutions of acetophenetidin, *p*-acetylaminophenol, phenobarbitone and salicylic acid were applied to a sheet of the DE20 paper, and the sheet was so placed in the micro-electrophoresis tank containing 0·2 N ammonia solution that only one edge made contact with the solution. After about 20 minutes, when the liquid had moved almost to the opposite edge of the paper, the sheet was removed, turned through a right angle and replaced in the tank so that two edges made contact with the ammonia solution. A constant current of 5 mA was then passed for 1 hour, after which the paper was removed and inspected, while wet, in light from a lamp emitting radiation of wavelength 254 mμ. The results of this experiment are shown in Fig. 1.

SEPARATION OF MIXTURE OF ACETOPHENETIDIN, SULPHACETAMIDE, QUININE AND PROMAZINE

For the separation of these basic and neutral compounds, the procedure used was exactly as described above, except that the ammonia solution was replaced by 0·1 M EDTA solution. The ionic strength of this solution is greater than that of the ammonia, so that the current was increased to 10 mA in order to achieve a potential of about 60 to 70 volts. The times allowed for chromatography and ionophoresis were 20 and 30 minutes, respectively. The results of this experiment are shown in Fig. 2.

DISCUSSION OF THE METHOD

The separation of acetophenetidin, *p*-acetylaminophenol, phenobarbitone and salicylic acid, as shown in Fig. 1, reveals two interesting features that are not immediately apparent. First, it can be seen that phenobarbitone moves faster than salicylate when subjected to ionophoresis on DE20 paper. According to Goldbaum,[4] salicylate moves faster than barbitone when subjected to ionophoresis on ordinary filter-paper. Secondly, it was noticed that, when the wet paper

was examined (after ionophoresis) in 254-mμ light, both phenobarbitone and *p*-acetylaminophenol showed a dark-purple absorbing area. When the paper was dried (with a hair-dryer) and again inspected in 254-mμ light, it was noticed that the *p*-acetylaminophenol displayed a pale-blue fluorescence. This phenomenon, which does not occur with ordinary filter-paper, serves as a useful adjunct in differentiating the phenol derivative from barbiturates.

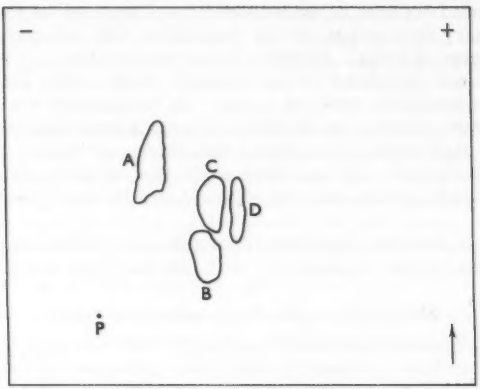

Fig. 1. Separation of mixture of drugs by chromatography in 0·2 N ammonia solution for 15 minutes and then ionophoresis (1 hour; 5 mA): A acetophenetidin; B, salicylate; C, *p*-acetylaminophenol; D, phenobarbitone. The mixture was applied at point P, and chromatography was by the ascending-solvent technique in the direction shown by the arrow

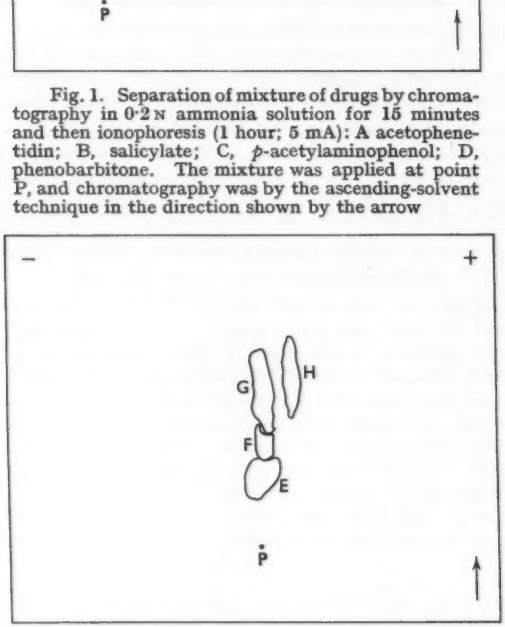

Fig. 2. Separation of mixture of drugs by chromatography in 0·1 M EDTA solution for 20 minutes and then ionophoresis (30 minutes; 10 mA): E, promazine; F, quinine; G, acetophenetidin; H, sulphacetamide. The mixture was applied at point P, and chromatography was by the ascending-solvent technique in the direction shown by the arrow

In the separation of the mixture of acetophenetidin and the basic compounds, chromatography in 0·1 M EDTA solution produced three components. These were promazine, quinine and a mixture of sulphacetamide and acetophenetidin. Resolution of sulphacetamide and acetophenetidin was achieved by ionophoresis in 30 minutes. For this separation, it was necessary to use a constant current of 10 mA to achieve a voltage adequate for separation of the two drugs. No adverse effects were noted from the resulting increase in the amount of heat produced.

Examination of the wet paper in 254-mμ light, as shown in Fig. 2, revealed that promazine gave a blue fluorescent spot and that both sulphacetamide and acetophenetidin gave dark-blue absorbing areas; quinine was not visible on the wet paper. After the paper had been dried (4 to 6 minutes with the hair-dryer), promazine displayed a brilliant yellow fluorescence, acetophenetidin a dark-blue absorbing area and sulphacetamide a pale-blue area. Quinine was detectable as a bright-blue fluorescent spot by holding the paper over the mouth of a bottle containing concentrated hydrochloric acid.

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Department of Forensic Medicine
University of Edinburgh
Teviot Place, Edinburgh

Harold V. Street
S. K. Niyogi

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DETERMINATION OF 2,6-DIAMINOPIMELIC ACID: A PAPER-CHROMATOGRAPHIC MODIFICATION OF WORK'S ACID - NINHYDRIN REACTION

For the quantitative determination of 2,6-diaminopimelic acid, Work described a sensitive and accurate method based on reaction with ninhydrin at an acid pH.[1] However, this method cannot be used for samples containing many other amino acids, as basic amino acids and cystine also produce considerable colour in the reaction. It was recommended that the components of such samples be first separated by paper chromatography and that the spot of diaminopimelic acid be cut out and subjected to a standardised procedure.[1,2] This modification was successfully used with samples containing relatively small numbers of amino acids,[2] but, when applied to samples containing large amounts of many interfering amino acids, it gave rather poor results. This was because, with most solvents, diaminopimelic and interfering basic amino acids are close to each other on paper chromatograms, and, in the solvent used by Rhuland, Work, Denman and Hoare,[3] the spots of diaminopimelic acid showed a strong tendency to "tail," which prevented their precise location. For use in these circumstances, the modified procedure described here was devised; it has been successfully applied to the determination of diaminopimelic acid in acid hydrolysates of bacteria.[4]

Method

Reagents—

All reagents should be of recognised analytical grade.
Acetic acid, 90 *per cent. v/v.*
Ninhydrin solution, 0·1 *per cent., in acetone.*

Procedure—

Apply the samples (containing 0·05 to 0·2 μmole of diaminopimelic acid) as spots or lines, depending on the amounts of other substances present, on strips of Whatman No. 1 chromatography paper. Allow chromatography by the descending-solvent technique to proceed for 12 to 18 hours at room temperature; use a mixture of methanol, water, 10 N hydrochloric acid and pyridine (80:17·5:2·5:10) as solvent.[3] Dry the chromatogram in a stream of air at room temperature for 30 to 60 minutes, dip it rapidly into the ninhydrin solution, dry as before for 5 to 10 minutes, and heat the dried strip in an oven at 105° C for 15 minutes. Cut out the spots of diaminopimelic acid (which have a characteristic olive-green colour and gradually fade to yellow), and cut them into small pieces. Place the pieces in a test-tube, add 5·0 ml of the acetic acid, and set aside for at least 30 minutes, with occasional shaking, to extract the colour. Transfer the yellow liquid to a centrifuge tube, and spin in a centrifuge at a slow rate to remove paper fibres. Measure the optical density of the supernatant solution against the acetic acid at 440 mμ with a spectrophotometer or an absorptiometer fitted with a filter transmitting at 430 mμ.

Prepare a blank solution by similarly treating a piece of paper cut from a portion of the chromatogram to which no sample has been applied; ensure that the piece of paper has an area

approximately the same as that containing the sample spots. Subtract the optical-density reading for the blank solution from that found for the sample, and calculate the amount of diaminopimelic acid present from a calibration graph preferably prepared on the same day.

Discussion of the method

A critical examination of the proposed procedure was carried out with use of synthetic diaminopimelic acid (obtained from Mann Research Laboratories Ltd.), which is a mixture of optical isomers. The absorption spectrum of the colour produced was, in the range 360 to 480 mμ, closely similar to that produced in Work's acid - ninhydrin reaction carried out with her reagent B at 37° C; it had an absorption maximum at 425 mμ. In the proposed method, 0·2 μmole of diaminopimelic acid produced a colour having an optical density of 0·32 at 440 mμ, whereas, from Work's results, it would be expected to have an optical density of 0·143 and 0·300 in the "standard" and "modified" ninhydrin procedures, respectively. The colour approximately obeyed Beer's law up to 0·2 μmole of diaminopimelic acid, but, if more than 0·4 μmole was present, deviation from Beer's law was pronounced. The blanks usually had optical densities of less than 0·02, so that small variations in the size of cut-out spots did not affect the results.

Results were reproducible, and, when the determinations were made under standardised conditions, the coefficient of variation at 0·2 μmole of diaminopimelic acid was less than 3 per cent. between experiments carried out on different days. The results varied by only a few per cent., whether the samples were applied to the paper as spots or as lines 5 cm long and whether chromatography was allowed to proceed for 8, 12 or 16 hours. The use of Toyo No. 50 or 51 filter-paper (average thickness, 0·26 or 0·18 mm) instead of Whatman No. 1 paper (average thickness, 0·13 mm) caused a variation of $\pm$9 per cent. in the intensity of colour. Variation in the composition of the solvent, *e.g.*, an increase or decrease of 30 to 50 per cent. in the amount of pyridine present, did not influence the final optical density.

Drying the paper in a stream of air is usually sufficient if carried out for 30 to 60 minutes, but it may be carried out for longer periods, as incomplete drying often tends to inhibit the colour development. Colour development is largely complete after 5 minutes at 105° C, but the intensity of colour continues to increase gradually, even after 15 minutes. When the strips of paper were heated for 15 minutes at 100°, 102·5°, 105°, 107·5° and 110° C, the relative intensities of the final colour were 91, 95, 100, 102 and 103, respectively. It is evident that variation of 2° to 3° C in temperature causes an error of only a few per cent. in the determination.

When ninhydrin solutions of concentration 0·05, 0·1, 0·2 and 0·3 per cent. were used, the relative intensities of colour were 91, 100, 105 and 109, respectively. The spots can be subjected to extraction from 15 minutes to 3 hours after development of colour. When they were extracted after being set aside for 24 hours in the dark, the final optical density was decreased by about 10 per cent. Extraction of colour is usually complete after 30 minutes, but can be continued for longer periods, as the extracted colour is stable for at least 24 hours at room temperature.

Cysteic acid, histidine, cystine, lysine and arginine produced colours having, respectively, 0·0, 2·4, 0·1, 5·5 and 1·7 per cent. of the intensity (at 440 mμ) of that produced by an equivalent amount of diaminopimelic acid. On paper chromatograms, cystine, lysine and arginine were clearly separated from diaminopimelic acid and did not interfere with the determination. Histidine travelled in close proximity to diaminopimelic acid and, when present in large amount, might interfere with the determination.

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Department of Bacteriology
Keio University School of Medicine
Shinjuku-Ku, Tokyo, Japan

Hiroshi Nikaido
Received *April 20th*, 1961

(Present address:
Institute for Protein Research,
Osaka University,
Osaka, Japan)

INDIRECT ULTRA-VIOLET SPECTROPHOTOMETRIC DETERMINATION OF SULPHUR DIOXIDE BY MEANS OF PLUMBOUS ION

BECAUSE of the unpredictability of the fuchsin - aldehyde colour reaction frequently used for determining sulphur dioxide (as liberated, for example, from thiodan[1] during the evaluation of residues of this insecticide), an alternative micro-analytical method for this gas is desirable; several approaches have been investigated. Although sufficiently sensitive, use of the sulphur dioxide for bleaching solutions of dyes, such as malachite green and an aniline blue, also suffered from unpredictable daily variation. The reduction of potassium permanganate in acid solution by the sulphur dioxide resulted in a satisfactory hypsochromic shift towards yellow, but sensitivity at the microgram level was poor; similar reductive bleaching of alkaline potassium permanganate also lacked photometric sensitivity. Direct ultra-violet evaluation of aqueous solutions of sulphite at an absorption maximum at 197 mμ was of intermediate sensitivity. Table I shows a comparison between the sensitivities of these methods.

TABLE I

SENSITIVITIES OF SOME SPECTROPHOTOMETRIC PROCEDURES FOR DETERMINING SULPHUR DIOXIDE

Reagent	Approximate weight of sulphur dioxide equivalent to 0·1 unit of optical density, μg
Malachite green	51 at 605 mμ
Aniline blue	Unstable
Acid potassium permanganate	32 at 440 mμ
Alkaline potassium permanganate	71 at 525 mμ 37 at 340 mμ
Aqueous solution of sulphur dioxide	50 at 197 mμ
Plumbous ion	8 at 208 mμ

The depletion of plumbous ion in an aqueous solution by precipitation is a promising method for sulphite-containing solutions or for gaseous (liberated) sulphur dioxide.[2] Plumbous ion absorbs strongly at 208 mμ (molecular extinction coefficient 8210), and this permits a sensitivity of 13μg of plumbous ion per 0·1 unit of optical density (equivalent to 8 μg of sulphur dioxide) to be attained. The lead precipitate is sufficiently insoluble in water at room temperature for plumbous ion not to be spectrophotometrically determinable from its saturated aqueous solution.

METHOD

To 1·00 ml of lead acetate solution (100 μg of Pb^{2+} per ml) in a calibrated centrifuge tube add, with thorough mixing, a measured aliquot of a freshly prepared aqueous standard solution of sodium sulphite. Spin in a centrifuge to separate the heavy precipitate, and then dilute to 5·0 ml with water. Measure the optical density of the final mixed solution (against water) from 250 to 190 mμ; use the optical density at 208 mμ to calculate the concentration of plumbous ion remaining in solution and hence, indirectly, the amount of sulphur dioxide added.

DISCUSSION OF THE METHOD

The decrease in optical density is proportional to the concentration of SO_3^{2-} ion present. A careful check made by adding fresh solid sodium sulphite directly to a solution of lead acetate established that, under the conditions specified, 2 moles of sulphur dioxide are needed to precipitate 1 mole of plumbous ion.

When this method is used for determining residues of thiodan,[1] interference will result from the presence of acid gases (such as hydrogen sulphide), which precipitate plumbous ion from aqueous solutions, and from accessory extractives that liberate acid or neutral water-soluble volatile substances absorbing strongly in the 250- to 190-mμ region. However, both sources of interference could be removed during the "cleaning-up" stages of the residue procedure.[1]

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DEPARTMENT OF ENTOMOLOGY
UNIVERSITY OF CALIFORNIA
RIVERSIDE, CALIFORNIA, U.S.A.

ROGER C. BLINN
FRANCIS A. GUNTHER
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THE DETERMINATION OF ZINC IN ZINC MANGANESE FERRITE AND OTHER FERRITES

THE manufacture of zinc manganese ferrite and other modern magnetic ferrites calls for careful control of composition. The determination of the zinc contents of these materials by traditional gravimetric methods based on separation as sulphide is tedious and calls for a fair degree of skill on the part of the operator. A precision polarograph of the type described by Gentry and Newson[1] has been used in this laboratory for some years for determining zinc in several types of ferrite, but this instrument is not commonly available, and typical commercial polarographs lack the precision necessary in the determination of the higher contents of zinc.

Several workers[2,3,4] have used ion-exchange separation and subsequent volumetric or photometric determination in an endeavour to simplify the procedure, and D. G. Timms, in a paper presented at the meeting of the Society for Analytical Chemistry on October 2nd, 1957, reported an attempt to determine the zinc by titration with disodium ethylenediaminetetra-acetate after complexation of iron and zinc with cyanide and then destruction of the zinc complex by formaldehyde. In our hands, Timms's method proved to be unreliable, and the ion-exchange methods, although sound, were dependent on factors such as the grade of resin available, rates of elution, etc. Attention was therefore turned to the possible use of a solvent-extraction procedure to separate zinc from the other constituents.

Mahlman, Leddicotte and Moore[5] showed that zinc could be extracted, as chlorozincate, into organic solvents by certain tertiary amines under conditions similar to those required for anion-exchange separation[6] and that separations possible by anion exchange might also be possible by amine extraction; such a procedure was devised by Scroggie and Dean[7] for determining zinc in non-ferrous alloys. Although ferric iron is extracted by this method, we have confirmed that, under the conditions used, ferrous iron is not.

When a 5 per cent. v/v solution of dioctylmethylamine in trichloroethylene was used to extract zinc from 3 to 4 N hydrochloric acid, recovery, as found by destroying the organic matter in the extract and subsequently titrating the zinc, was quantitative. However, when attempts were made to simulate the ion-exchange procedure by re-extraction of the zinc into 0·005 N hydrochloric acid or water, the amounts of zinc recovered were only about 10 per cent. of those added. It was then found that, when sufficient ethanol to form a single phase was added, zinc in the organic extract could be determined by direct titration with disodium ethylenediaminetetra-acetate without further separation. Recovery by this method was tested over the range 10 to 25 mg of zinc in the presence of up to 100 mg of iron; the error did not exceed $\pm$0·1 mg.

METHOD

REAGENTS—

Dioctylmethylamine solution, 5 per cent. v/v in trichloroethylene.
Ethanol, 95 per cent.
Ascorbic acid.
Disodium ethylenediaminetetra-acetate, 0·01 M.
Eriochrome black T indicator solution, 0·05 per cent. in ethanol.
Ammonia solution, sp.gr. 0·880, diluted (1 + 1).
Hydrochloric acid, sp.gr. 1·18, diluted (1 + 1).

PROCEDURE—

Transfer the sample, containing 10 to 25 mg of zinc in 10 ml of 2 N hydrochloric acid, to a 100-ml separating funnel, and add excess of solid ascorbic acid to reduce ferric iron (about 200 mg of the acid should be added in excess of that needed to destroy the yellow colour of ferric iron). Add 5 ml of the diluted hydrochloric acid and then 20 ml of dioctylmethylamine solution. Insert a stopper in the neck of the funnel, shake for 3 minutes, allow the layers to separate, and run the organic layer into a second separating funnel. Extract the aqueous layer with a further 20 ml of dioctylmethylamine solution for 3 minutes, combine the organic extracts, and shake them for 1 minute with 20 ml of the diluted hydrochloric acid to remove any traces of nickel, cobalt, copper, etc., possibly co-extracted with the zinc.[5] Run the organic layer into a 350-ml conical flask, and add 100 ml of 95 per cent. ethanol and then 25 ml of the diluted ammonia solution (this should give a slight excess of ammonia). Determine the zinc by titration with 0·01 M disodium ethylenediaminetetra-acetate in the presence of 2 ml of Eriochrome black T solution as indicator.

Results

The proposed method is rapid, simple and requires no special skill; it has been applied to several zinc manganese ferrites and zinc ferrites (see Table I) and should be equally applicable to the determination of zinc in other ferrite materials containing cobalt, nickel or copper.

Table I

Zinc contents found in various ferrites

Sample No.	Manganese content, as MnO, %	Iron content, as Fe_2O_3, %	Zinc found, as ZnO, by—proposed method, %	Zinc found, as ZnO, by—alternative method, %
1	16	76	6·9	7·1*
2	15	70	14·1	14·1*
3	—	43	56·2	56·2†
4	—	34	65·1	64·9†

* Result found gravimetrically after separation as sulphide.
† Result found polarographically.

We thank Dr. J. A. M. van Moll, Head of the Material Research Laboratory, and the Directors of The Mullard Radio Valve Company Ltd. for permission to publish this Note.

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Material Research Laboratory
The Mullard Radio Valve Company Ltd.
New Road, Mitcham Junction, Surrey

T. R. Andrew
P. N. R. Nichols
Received *April 24th*, 1961

THE APPLICATION OF A SPECTROPHOTOMETRIC ANALYTICAL TECHNIQUE IN A KINETIC STUDY

If a solution containing two mutually inert absorbing entities (A and B) obeys Beer's law, analysis can easily be carried out by measuring the optical densities, $E_{\lambda 1}$ and $E_{\lambda 2}$, at any two convenient wavelengths, λ_1 and λ_2. The molarities of A and B can be found by solving the simultaneous equations shown below, in which the symbols used have the usual meanings.

$$E_{\lambda 1} = \epsilon_A^{\lambda 1} A l + \epsilon_B^{\lambda 1} B l$$
$$E_{\lambda 2} = \epsilon_A^{\lambda 2} A l + \epsilon_B^{\lambda 2} B l$$

However, in certain circumstances, *e.g.*, in kinetic studies, it may be desirable to obtain measurements of E, ϵ_A and ϵ_B at a series of wavelengths in order to check that only two absorbing species are present. The concentrations of A and B can then be deduced from a graph of either $E/\epsilon_A l$ or $E/\epsilon_B l$ against the ratio of the extinction coefficients at each wavelength. The graph should be linear over the whole range of wavelengths investigated; if it is significantly non-linear over any portion, then the presence of interfering absorbing species is indicated. This procedure therefore gives an immediate visual check on the self-consistency of the results and can also assist in their quantitative interpretation. This approach was recently applied during a kinetic study of the photodecomposition of a peroxide. In this instance, the reactant and one product absorbed and there was no thermal reaction.

Aqueous 1 per cent. solutions of diethyl peroxide were irradiated, and chemical tests indicated that ethanol and acetaldehyde were the only products. However, accurate quantitative determinations on the mixtures obtained were not easy, particularly as many of the experiments were terminated when the concentration of peroxide had been decreased by as little as 0·01 M. All analytical methods found in the literature were unsatisfactory, owing to lack of sensitivity or poor reproducibility (often because of interference from one of the other two components) or both.

The chemical methods had the further disadvantage that the experiment had to be terminated in order to make the determinations. However, an accurate and convenient quantitative determination was devised as described below.

The ultra-violet spectra of the peroxide and acetaldehyde in aqueous solution (see Fig. 1) were measured between 2400 and 3400 A in the respective concentration ranges 0·02 to 0·15 M and 0·005 to 0·05 M in order to check that Beer's law was obeyed at all concentrations encountered in the work. Fig. 2 shows the absorption spectrum after the peroxide had been partly decomposed. These results were then analysed as follows.

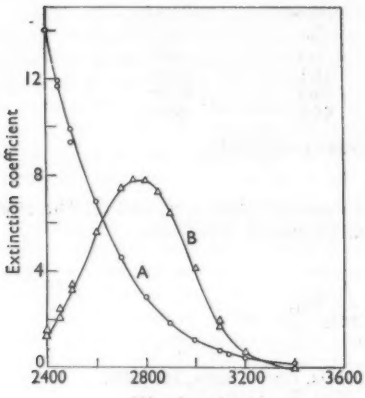

Fig. 1. Absorption spectra of diethyl peroxide (curve A) and acetaldehyde (curve B) in aqueous solution

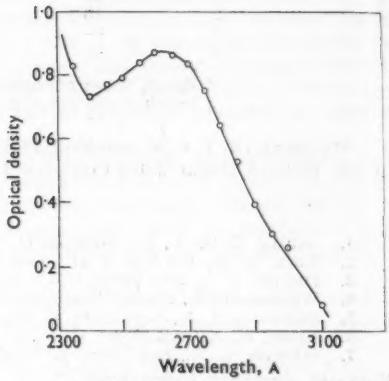

Fig. 2. Absorption spectrum of partly decomposed diethyl peroxide

Let ϵ_A and ϵ_P be the extinction coefficients of the acetaldehyde and peroxide, respectively, at any fixed wavelength, and let E_0 be the initial optical density, E_n the optical density after reaction for n minutes, P_0 the initial molarity of peroxide and P_n and A_n, respectively, the molarities of peroxide and acetaldehyde after reaction for n minutes. If kA_n moles of acetaldehyde are formed for each mole of peroxide destroyed, and the length of the cell is 1 cm, then—

$$E_0 = \epsilon_P P_0 l \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

$$E_n = \epsilon_P P_n l + \epsilon_A A_n l \quad .. \quad .. \quad .. \quad .. \quad (2)$$

$$P_0 - P_n = kA_n \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

Substitution in equation (2) for P_n from equation (3) gives—

$$E_n = \epsilon_P l(P_0 - kA_n) + \epsilon_A A_n l$$
$$= E_0 - kA_n\epsilon_P l + \epsilon_A A_n l$$

from which it can be seen that—

$$A_n = \frac{E_0 - E_n}{l(k\epsilon_P - \epsilon_A)}.$$

Division of this equation by P_0 from equation (1) gives—

$$\frac{A_n}{P_0} = \frac{(1 - E_n/E_0)}{(k - \epsilon_A/\epsilon_P)}.$$

Let A_n/P_0 be f; then rearrangement of this expression gives—

$$\epsilon_A/\epsilon_P = \frac{E_n}{fE_0} - \frac{1}{f} + k \quad .. \quad .. \quad .. \quad .. \quad (4)$$

Thus, from a graph of the ratio of the extinction coefficients (ϵ_A/ϵ_P) against the ratio of optical densities (E_n/E_0) at each wavelength can be derived—

(*i*) the slope, which is 1/f, *i.e.*, P_0/A_n,

(*ii*) the intercept when E_n/E_0 is zero, which is $k - 1/f$, *i.e.*, $-P_n/A_n$, and

(*iii*) the intercept when ϵ_A/ϵ_P is zero, which is $1 - kf$, *i.e.*, P_n/P_0.

Fig. 3 shows some graphs of ϵ_A/ϵ_P against E_n/E_0 for this particular system. The technique was to irradiate for n minutes and then measure the optical density (E_n) at five wavelengths between 2400 and 3400 A, thereby obtaining points on one of the lines on the plot. Irradiation was then continued for a further period, after which optical densities were again measured at the same five wavelengths, and so on. The lines in Fig. 3 therefore represent three consecutive periods of irradiation. It is often a feature of studies of this type that secondary reactions involving the products occur, complicating the determinations and the kinetics. These effects generally become progressively more serious as larger portions of the compound being studied are decomposed. The consistent linearity of the graphs in Fig. 3 indicates that, in this system, other absorbing species were not created in significant amounts.

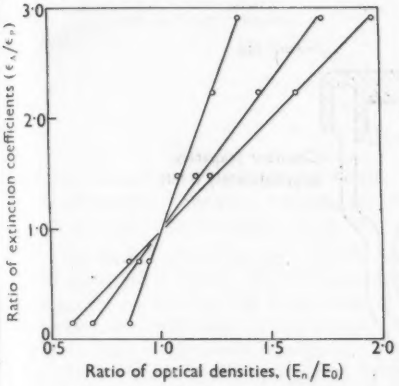

Fig. 3. Graphs of ϵ_A/ϵ_P against E_n/E_0 for three consecutive periods of irradiation

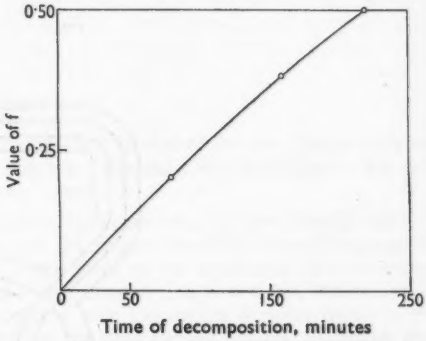

Fig. 4. Graph of values of f calculated from the slopes of the lines in Fig. 3 against time of decomposition

One further derivation can be made from equation (4), *viz.*, when ϵ_A/ϵ_P and E_n/E_0 are both equal to 1, *k* also is equal to 1. If, therefore, the assumptions are correct and if equimolar amounts of acetaldehyde are formed from the peroxide, then all lines must pass through the point (1,1). All the lines plotted in Fig. 3 fall slightly below this point, owing to loss in the grease on the tap of the reaction cell of a small fraction of the acetaldehyde formed.

Fig. 4 is a graph of three values of f (obtained from the slopes of the lines in Fig. 3) against n, the time of decomposition. The graph is almost linear, which indicates that the rates of decomposition of the peroxide and production of acetaldehyde were approximately constant during the 4-hour experiment. It should be emphasised that such a graph is worth-while, essentially because, as well as permitting quantitative determination, it simultaneously provides a rapid check that only the two species considered in the mathematical treatment are present in any mixture.

If a solution free from interference, *i.e.*, known to contain only two absorbing entities is to be analysed, then the usual method of solving the two simultaneous equations given in the introduction is more accurate. The two wavelengths used should be properly chosen; one would avoid choosing a pair of wavelengths at which the extinction coefficients of the two species were about equal, since readings at these wavelengths would have low statistical weight.

IMPERIAL OIL LTD.
P.O. BOX 3022
SARNIA, ONTARIO, CANADA

G. A. HOLDER
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Apparatus

A SIMPLE BY-PASS INJECTOR FOR GAS CHROMATOGRAPHY

In the development of a suitable instrument for the gas-chromatographic analysis of mixtures of permanent gases, it was desirable to be able to inject small volumes of standard gases into the carrier-gas stream. One common method of doing this is to use an Agla-type pipette, the needle of which is inserted into the carrier gas through a serum cap. However, reproducible results could not be obtained when this technique was used with an instrument designed to operate at a carrier-gas pressure of approximately 12 lb per sq. inch above atmospheric. Possible reasons for this could be (*a*) loss, around the needle, of carrier gas carrying part of the sample during the time required for the injection or (*b*) transfer of carrier gas to the pipette before the sample is expressed.

These difficulties can be avoided by employing a by-pass sample injector, in which the serum cap is positioned in a sampling loop that can be operated at or near atmospheric pressure. (The term "by-pass sample injector" has been adopted following the International Union of Pure and Applied Chemistry's recommendations on nomenclature and presentation of data in gas chromatography.[1]) A number of by-pass injectors have been described,[2,3,4,5] and sampling valves operating on similar principles are also commercially available.

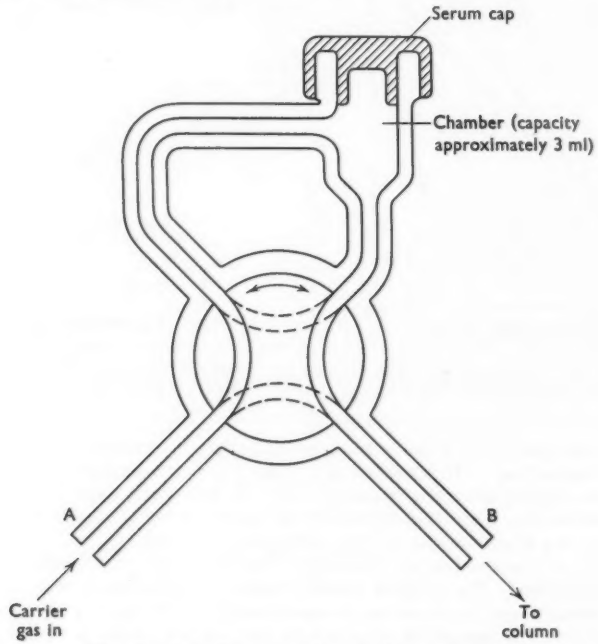

Fig. 1. Sampling valve for gas chromatography

The by-pass injector shown in Fig. 1 is, however, simpler in construction and operation than any that we have yet seen described. It is made of glass and consists of a spring-loaded four-way tap with a small chamber and fitted serum cap inserted in a loop between two of the ways. The chamber has a capacity of approximately 3 ml and is an integral part of the gas-flow system and not merely a pressure chamber opening from it. The four-way taps used for these sampling valves are commercially available.

Method of operation

The tap is turned to allow the carrier gas to pass directly from A to B, and the gas pressure in the chamber is decreased almost to atmospheric by inserting a hypodermic needle through the

serum cap. The gas sample, contained in an Agla-type syringe pipette, is injected into the chamber in the usual way and is then transferred to the chromatographic column by rotation of the tap.

This by-pass injector has been used for fuel-gas mixtures, such as town gas, water gas and Fischer - Tropsch synthesis and residual gases containing hydrogen, oxygen, nitrogen, carbon monoxide, carbon dioxide, methane and small amounts of higher hydrocarbons. It is simple to make, rapid and easy to use and requires no volume calibration; further, it permits gas samples to be quantitatively transferred to a chromatographic column in a reproducible manner. The volumes of such samples can be varied continuously in the range 0 to 2 ml, and standard samples of known composition can be prepared in the chamber by injecting successive volumes of pure gas.

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Department of Scientific and Industrial Research
Warren Spring Laboratory
Gunnels Wood Road
Stevenage, Herts.

P. J. Kipping
P. G. Jeffery
Received *May* 31st, 1961

Book Reviews

The Identification of Organic Compounds: A Manual of Qualitative and Quantitative Methods. By Stig Veibel, Dr.Phil. Fifth Edition. (Second English Edition.) Pp. xvi + 426. Copenhagen: G. E. C. Gad Publisher. 1961.

This second edition of Dr. Veibel's book contains 80 more pages than the first English edition, which appeared in 1954. The first chapter briefly reviews the classical methods of purifying organic substances: no reference is made here, or elsewhere in the book, to the separation of mixtures of the type commonly used in analytical exercises. The next chapter contains a full description of the detection of elements in organic compounds, together with the details of some methods for their determination; the specialised techniques required for determining carbon, hydrogen and oxygen are not discussed, but references to methods are given.

The burden of the book is a discussion, in 340 pages, of the detection and determination of functional groups. Descriptions of methods of determination account for about one third of the material, and, although this generally falls outside the scope of undergraduate teaching in organic chemistry in this country, it would be of value to the research worker. After a discussion of preliminary tests, the characteristics of each functional group are set out in separate sections, a method that the reviewer finds superior to attempts at tabulation based on elemental composition or solubility. More information about typical physical properties should have been included in each section, particularly since tables of physical constants are not included: this omission will, however, ensure that students refer to the general literature.

In a few instances, practical details were found to be inexplicit, for example, reduction of nitro-compounds as described on p. 335; on the other hand, details are given for preparing a wide range of derivatives for characterisation, and useful comments are made about variation of conditions to meet different reactivities of the functional group. The inclusion of details of chromatographic procedures and of determination by non-aqueous titration is a valuable feature. The author has listed (p. 60) some of the principal works of reference to methods of absorption spectroscopy, and indeed characterisation of organic compounds without their application must be an academic exercise, albeit an exercise of great value in teaching. From an educational point of view, the inclusion of too many colour tests for functional groups seems undesirable to the reviewer, and he would fault the book on this score, despite the critical attitude the author takes to tests of this kind, as evidenced by the comments on characterising amines on p. 258.

The book is well produced in attractive type and has an effective index. In order that it may attract readers, it is to be hoped that the price of this edition will be in the same range as the previous one at £2 6s. 6d.

D. C. Ayres

TREATISE ON ANALYTICAL CHEMISTRY. Edited by I. M. KOLTHOFF and PHILIP J. ELVING, with the assistance of ERNEST B. SANDELL. Part I. Theory and Practice. Vol. 1. Pp. xxvi + 809. Vol. 2. Pp. xx + 811–1308. New York and London: Interscience Publishers Inc. 1959 (Vol. 1) and 1961 (Vol. 2). Price (Vol. 1) $16.00; 120s. Price (Vol. 2) $16.00; 120s.: (subscribers to whole series) $14.00; 105s.

This series represents a most ambitious project designed to cover all aspects of analytical chemistry. It is proposed to divide the subject into three parts, *viz.*, I. Theory and Practice II. Analytical Chemistry of the Elements and III. Analysis of Industrial Products. Part I, on Theory and Practice, is already published and is in two volumes. Part II is to be divided into three volumes dealing with (*a*) the determination of the elements in inorganic form, (*b*) the determination of the elements in organic form or as organic functional groups and (*c*) the systemic treatment of the detection of the elements and their functional groups.

The first volume on Theory and Practice has an impressive range of authors from the leading Universities of the U.S.A., and the subjects covered comprise many of the most important aspects of theoretical analytical chemistry. E. B. Sandell contributes a short section on the objectives, functions and limitations of analytical chemistry, which includes errors in chemical analysis, accuracy and precision and principles and methods of sampling. The rest of this volume is made up of application of general principles. The atomic theory in all its aspects from the viewpoint of modern physical chemistry is fully presented, and this includes modern methods for the determination of atomic weights. Chemical equilibrium and the thermodynamics of reactions are detailed. Electrode potentials, pH, acid - base concepts and protolysis curves are described. Complexation reactions, oxidation - reduction, titration curves, solubility and precipitate formation, including equilibria reactions, are dealt with in great detail. Some 800 pages are required for the above material, which obviously covers a considerable portion of the whole field of physical chemical knowledge.

The second volume deals with reactive groups as reagents, the principles and technique of separations (including mechanical methods), diffusion, electrophoresis and distillation and contains a good chapter on solution of samples. The same fundamental treatment is given to these subjects, and in the main the presentation is clear and logical. The treatment is certainly more than sufficient for the analyst, but it is doubtful whether it would be wide and detailed enough for a student to finals standard. The subject is by no means complete, and we are promised in the preface that future volumes will be concerned with instrumentation, measurement, optical methods, etc. It may well be that in making this attempt to segregate analytical chemistry from other academic aspects of chemistry, the editors have indeed shown how impossible the task becomes. If, however, this results in the full realisation that analytical chemistry is a fundamental integral of general chemical knowledge, then projects such as these will have achieved some purpose.

Although, to-day, analytical chemistry is more respectable in academic circles than formerly, there is still a reluctance on the part of University authorities to give it its rightful place—as witnessed by the all too few Chairs on the subject in this country.

Volumes such as these serve a useful purpose in showing that analytical technique is no longer a matter of trial and error and inspiration, but may be developed deliberately from fundamental concepts.

R. F. MILTON

TREATISE ON ANALYTICAL CHEMISTRY. Edited by I. M. KOLTHOFF and PHILIP J. ELVING, with the assistance of ERNEST B. SANDELL. Part II. Analytical Chemistry of the Elements. Vol. 3. Pp. xviii + 380. New York and London: Interscience Publishers Inc. 1961. Price (single volume) $13.25; 100s.: (subscribers to whole series) $12.00; 90s.

This volume covers the analytical chemistry of six elements, *viz.*, cadmium, copper, magnesium, mercury, tin and zinc. Each element is dealt with in considerable detail under such headings as Occurrence in Nature; Production; Physical and Chemical Characteristics, Separation and Isolation; Qualitative Identification and Quantitative Determination. Under the final heading is given a listing of the various group methods, *e.g.*, gravimetric, titrimetric, polarographic and photometric; then the analysis of alloys and compounds and finally the determination of the metal in specific materials. The section ends with a description of recommended laboratory procedures and finally a list of references—up to 400 for each element.

It is my opinion that this treatment is completely inadequate and illustrates the difficulty of attempting to survey everything about one element within a small compass. For instance, some 37 pages are devoted to copper, and, as a result, the natural occurrence is dealt with in

2½ lines, the methods of production in less than 1 page and chemical properties (including the various reactions important in the determination of the element) in 2 pages. Titrimetric methods are dismissed in 2 pages, and photometric methods in 1½. Determination of copper in specific materials is described in a very sketchy manner, and I looked in vain for any single determination described in detail such that a practising analyst could use.

Determinations of zinc and mercury are rather more fully described in some 65 and 98 pages, respectively. But even in these instances the information given is very limited and could at the best be a starting-off point for the reader to seek further information on the subject elsewhere. It is because of these considerations that I am at a loss to decide for what particular readership this volume is written. Certainly not the practising analyst, who would require very much more information than that given before he could ever attempt to use any of the methods recommended, and certainly not for the student in chemistry, who needs to know about the elements. The book then becomes merely an index of possible methods, with a certain background make-weight of information of a general nature. Maybe the references are more valuable. I hope to find this out in due course.

R. F. MILTON

GAS CHROMATOGRAPHY. By ERNST BAYER. Pp. xii + 240. Amsterdam, London, New York and Princeton: Elsevier Publishing Co.; London: D. Van Nostrand Co. Ltd. 1961. Price 25s.

This book is a welcome addition to the range—still somewhat limited—of text-books on gas chromatography. It provides the analyst with a sound practical guide to the use of gas chromatography, whether he is a newcomer to the technique or has already had some experience in its use.

The subject-matter is divided logically into six chapters. The first two chapters—Introduction and Theoretical Treatment—provide the historical and theoretical background necessary for a proper understanding of the subject and an adequate appreciation of its capabilities. The dependence of the HETP on gas velocity would have been brought out rather more clearly, however, if the short form of the van Deemter equation had been written in the more usual way, *viz.*, $H = A + B/u + Cu$, rather than $H = A + B + C$.

Chapter 3 deals with the design of columns and the behaviour of various stationary phases, both solid and liquid; the disadvantages of using spiral or horizontal columns are stressed. The influence of the solid support on the behaviour of liquid columns is discussed, although perhaps more space could have been devoted to methods of minimising this. The selectivity of various liquids is mentioned, and there is a useful Table indicating suitable stationary-phase liquids for typical separations. Columns of high resolution, including capillary columns, are also described. The maximum working temperatures given for various stationary phases on page 28 are too high when high-sensitivity detectors are used, and this should have been stated; the Table should in fact be read in conjunction with the author's statement on pp. 7 and 52 that thermal conductivity is the most frequently used method of detection. This statement is itself becoming less and less valid at the present time.

Chapter 4 indicates the various means available for controlling the flow of carrier gas, introducing the sample, detecting the emergence of sample components from the column, controlling the temperature of the column and collecting separated fractions.

Chapter 5, which deals with Practical Applications, is the longest and probably the most useful chapter in the book; it is mercifully subdivided into two sections on the principles of qualitative and quantitative analysis, followed by nineteen sections, each devoted to a particular class of analysis. Each section is further subdivided to deal with specific analyses. It is impossible to review this chapter in detail; it is so comprehensive in scope that beginners and experienced chromatographers alike can be sure of finding therein assistance in solving the unusual analytical problems that are bound to come their way.

Chapter 6 is an extension into greater detail of the "detectors" section of Chapter 4 and explains the principles on which each type operates. I must, however, disagree with the author's statement on p. 175 that flame detectors cannot be used for samples of chlorinated hydrocarbons because these extinguish the flame. I have many times used a Scott flame-thermocouple detector and obtained *positive* peaks in all cases—even with carbon tetrachloride; the sensitivity is admittedly low, but this merely means that calibration is necessary with such samples.

Reference should be made to one or two rather curious statements in this book. On p. 19, the author states that "Columns with greater diameters (than 2 to 10 mm) are not advisable, since the higher flow rate of the carrier gas . . . requires excess pressures of several atmospheres. . . ."

This seems to disregard the fact that rate of flow is proportional to cross-sectional area; the optimum *linear* rate of flow is not changed by increasing the column diameter, so that the pressure gradient also should be independent of column diameter. Also, on p. 53, the high temperature coefficient of thermistors is described as "detrimental," yet it is to this property that the thermistor detector owes its sensitivity. Presumably, on p. 56, chart speeds of 60 to 600 metres per hour should read 60 to 600 mm per hour.

Chapter 6 is followed by twenty-two Tables of selectivity and retention data collected from the literature and 420 references.

I regard this book as remarkably good value for its content of useful information and also its dimensions, which facilitate quick and easy reference. B. A. ROSE

Publications Received

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SEPARATIONS BY SOLVENT EXTRACTION WITH TRI-n-OCTYLPHOSPHINE OXIDE. By J. C. WHITE and W. J. ROSS. Pp. vi + 56. Washington, D.C.: National Academy of Sciences—National Research Council. 1961. Price 75 cents.
Nuclear Science Series: NAS—NS-3102. Radiochemical Techniques.

LOW-LEVEL RADIOCHEMICAL SEPARATIONS. By T. T. SUGIHARA. Pp. vi + 34. Washington, D.C.: National Academy of Sciences—National Research Council. 1961. Price 50 cents.
Nuclear Science Series: NAS—NS-3103. Radiochemical Techniques.

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QUANTITATIVE CHEMISTRY: A LABORATORY TEXT. By JURG WASER. Pp. x + 286. New York: W. A. Benjamin Inc. 1961. Price $3.25.

RESEARCH & DEVELOPMENT FOR INDUSTRY. Volume I, No. 1, September 1961. Edited by D. M. DESOUTTER. Pp. 160. London: British Aviation Publications. 1961. Annual Subscription 35s.: $5.25. Single copies 2s. 6d.